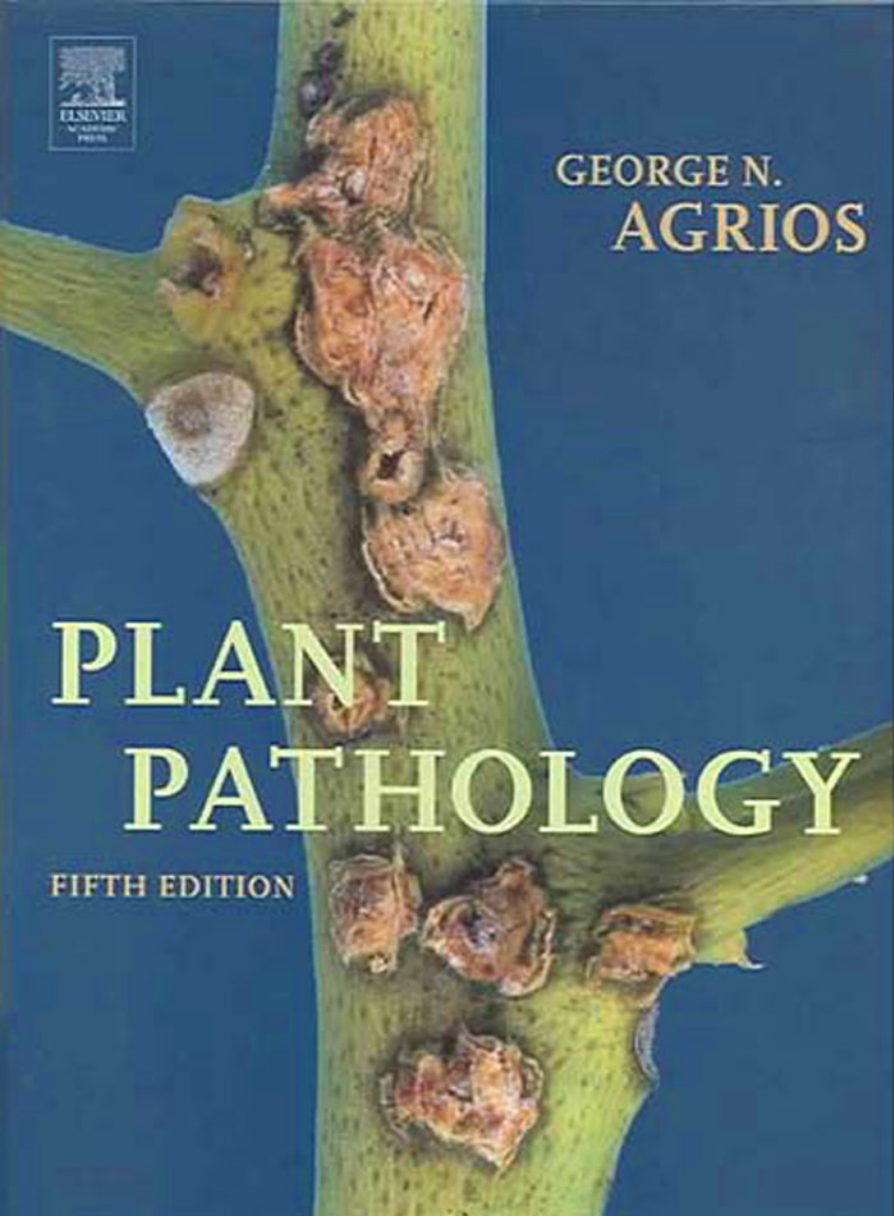
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Fifth Edition

PLANT PATHOLOGY

Fifth Edition

PLANT PATHOLOGY

GEORGE N. AGRIOS
Department of Plant Pathology
University of Florida

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This, the 5th and probably the last edition of *Plant Pathology* by me, is dedicated:

To the memory of my parents, Nikolas and Olga, who, in spite of their limited education, sacrificed everything to give me the most and best education possible.

To the memory of Dr. Walter F. Buchholtz, my major professor at Iowa State University, who challenged me before I had even taught my first lecture to "write my own textbook on Plant Pathology".

To my sisters, Dimitra and Evangelia, who have been there for me forever and who also sacrificed some of their interests for my benefit.

To my wife, Annette, whose love and support have been the most precious things to me throughout our life together, and who helped me in many facets of preparation of this and of previous editions of *Plant Pathology*.

To my daughters-in-law, Betsy and Vivynne, who, by joining our family, added beauty, love, enjoyment, and four wonderful grandchildren.

Finally, to Mark and Maximos, our youngest grandchildren, who, someday, when they read their names in the book, may be reassured of "Granpa's" love for them, and may feel proud of their grandfather.

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PLANT DISEASES CAUSED BY FUNGI

Preface

Since the appearance of the 1st edition of *Plant Pathology* in June 1969, tremendous advances have been made both in the science of plant pathology and in the publishing business. New information published in the monthly plant pathological and related biological journals, as well as in specialized books and annual reviews, was digested and pertinent portions of it were included in each new edition of the book. The worldwide use of the book, in English or in its several translations, also created a need to describe additional diseases affecting crops important to different parts of the world. There has been, therefore, a continuous need to add at least some additional text and more illustrations to the book with as little increase in the size of the book as possible. Fortunately, through the use of computers, tremendous advances have been made in the publishing business, including paper quality and labor costs and, particularly, in the reproducibility and affordability of color photographs and diagrams. Plant diseases and plant pathology come alive when illustrated in full color and it has been the author's dream to have all the figures in color. Add to these advances the interest of the author and of the publishers to spare no effort or expense in the production of this book and you have what we believe is the best book possible for the effective teaching of plant pathology at today's college level worldwide.

To begin with, "Plant Pathology, 5th edition" provides each instructor with all the significant new developments in each area and gives the instructor choices in the type and amount of general concepts material (Chapters 1–9) and of specific diseases (Chapters 10–16) he/she will cover. Each chapter begins with a fairly detailed, well-organized table of contents that can be used by students and instructors as an outline for the chapter. The instructor can also use it to cover parts of it in detail in class while some of the topics are covered briefly and others are assigned to the students as further reading. Each student, however, has all the latest material, well organized and beautifully illustrated, available in a way that is self-explanatory and, with the complete glossary provided, can be understood with minimal effort.

Instructors will have an even greater choice in the kinds of specific diseases one would use in a specific area of the country or of the world where one teaches. While one may want to include the teaching of potato late blight, apple scab, wheat rust, bacterial soft rot, root knot, and some other diseases of general interest, one often also wants to cover diseases of particular interest in the region, both because of their regional importance and because of their availability locally for further study in the classroom and the laboratory. This edition makes this possible by covering and illustrating in full color a wide variety of diseases, some of which are important to the grain plains of the Midwest and the northwestern United States, others to the fruit- and vegetable-producing Pacific and northeastern states, others to the

cotton-, peanut-, tobacco-, rice-, and citrus-vegetable producing southern states, and so on. A special effort has also been made to describe and to fully illustrate in full color several diseases of tropical crops important in different parts of the world, such as rice in the Far East, beans in Central and South America, cassava, cacao, and sorghum in Africa, and tropical fruits such as citrus, papaya, coconut, and coffee in the Americas, and so on. Instructors can pick and choose to study, in the classroom and, if possible, in the laboratory, whatever diseases of whichever crops they deem most significant for the particular area and for the ever-shrinking world we all live in.

The overall arrangement of this edition is similar to that of previous editions. However, all aspects of the book have been thoroughly updated and illustrated. Newly discovered diseases and pathogens are described, and changes in pathogen taxonomy and nomenclature are incorporated in the text. Changes or refinements in plant disease epidemiology and new approaches and new materials used for plant disease control are discussed. The chapters on diseases caused by prokaryotes (bacteria and mollicutes), especially the one on diseases caused by plant viruses and viroids, have been revamped due to the large amount of new information published in recent years about such pathogens and diseases. And in all cases, partial tables of contents have been added to each chapter and to its main subdivisions for better clarity and understanding of the arrangement and inclusion of the topics in the appropriate subdivisions. A new feature that has been added to the book is the presentation of a number of topics of special interest in separate boxes. In these, the various topics are approached from a different angle and highlight the importance of the topic whether it has historical, political, or scientific significance. Special attention has also been given to highlighting the historical developments in plant pathology and the scientists or others who contributed significantly to these developments.

As in other recent editions, much of the progress in plant pathology has been in the areas of molecular genetics and its use in developing defenses in plants, against pathogens. Discoveries in basic molecular genetics, particularly discoveries in how plants defend themselves against pathogens and in the development of mechanisms to produce disease resistant plants, receive extensive coverage. It is recognized that some of the included material in Chapters 4 (Genetics of Disease), 5 (How Pathogens Attack Plants), and 6 (How Plants Defend Themselves against Pathogens) may be both too much for students taking plant pathology for the first time and somewhat difficult to follow and comprehend. However, the importance of that material to the future development of plant pathology as a science and its potential future impact on control of plant diseases is so great that its inclusion is considered justified if only to expose and initiate the students to these developments.

There are numerous colleagues to whom I am indebted for suggestions and for providing me with numerous slides or electronic images of plant disease symptoms or plant pathology concepts that are used in the book. Their names are listed in the legend(s) of the figures they gave me and in the list of "Photo Credits." I would particularly like to express my sincere appreciation and thanks to Dr. Ieuan R. Evans of the Agronomy Unit of the Alberta Agriculture, Edmonton, Alberta, Canada, who, as editor of the slide collection of the Western Committee on Plant Disease Control, provided me with hundreds of excellent slides and permission to use them in the book. I also thank Dr. Wen Yuan Song for reviewing the chapter on "How Plants Defend Themselves against Pathogens." Finally, I again thank publicly my wife Annette for the many hours she spent helping me organize, copy, scan, and reorganize the many slides, prints, and diagrams used in this book. Not only did she do it better, she also did it faster than I could have done it.

George N. Agrios
July 2004

Photo Credits

The need for high-quality photographs to include in this book necessitated the request of appropriate photographs from colleagues around the world. All of them responded positively and I am very thankful to all of them. I am particularly indebted to the following individuals and organizations who, although I was asking from them one or a few photographs, sent me those plus all the related or other pertinent photographs that I might want to use in the new edition of the book. Moreover, several of them offered to give me any other photographs they had and which I might want to use.

I am particularly indebted to Dr. Ieuan R. Evans of the Agronomy Unit, Agriculture, Food, and Rural Development of Alberta, Canada, for providing me with several hundreds of slides put together by the Western Committee on Plant Diseases (WCPD) for general use for educational purposes. Those contributing slides through the WCPD include P. K. Basu, Agriculture Canada, Ottawa, Ontario; J. G. N. Davidson, Agric. Canada, Beaverlodge, Alberta; P. Ellis, Agric. Canada, Vancouver, British Columbia; I. R. Evans, Agric. Canada, Edmonton, Alberta; G. Flores, Agric. Canada, Ottawa, Ontario; E. J. Hawn, Agric. Canada, Lethbridge, Alberta; R. J. Howard, Alberta Agriculture, Brooks, Alberta; H. C. Huang, Agriculture Canada, Lethbridge, Alberta; J. E. Hunter, NYAES, Geneva, New York; G. A. Nelson, Agriculture Canada, Lethbridge, Alberta; R. G. Platford, Manitoba Department of Agriculture, Winnipeg, Manitoba; and C. Richard, Agriculture Canada, Sainte-Foy, Quebec.

I am equally indebted to Dr. Gail Wisler, Chair, Plant Pathology Department, University of Florida, for allowing me to use whatever slides of the departmental Plant Disease Clinic would be useful in illustrating the book. Since all of the slides were stamped with the name of Dr. G. W. Simone, and some of them were undoubtedly taken by him while he was an Extension Plant Pathologist in charge of the Plant Disease Clinic in the Department, now retired, I would like to express my thanks to Dr. Simone also.

I am also thankful to several other organizations that gave me permission to use many of their photographs and offered to give me any others I might need. They include the Extension Service of the University of Florida Institute of Food and Agricultural Sciences (UF/IFAS), the American Phytopathological Society, and several United States Department of Agriculture (USDA) Laboratories. I am particularly thankful to the USDA Forest Service along with the University of Georgia who, through "Forestry Images" and "Bugwood Network," provided me with several images of forest tree diseases.

I am particularly indebted to the following colleagues, listed alphabetically, each of whom gave me numerous slides or electronic images and offered to give me as many more of their photographs as I needed: Dr. Eduardo Alves, Federal University of Lavras, Brazil; Dr. Mohammad Babadoost, University of Illinois; Dr.

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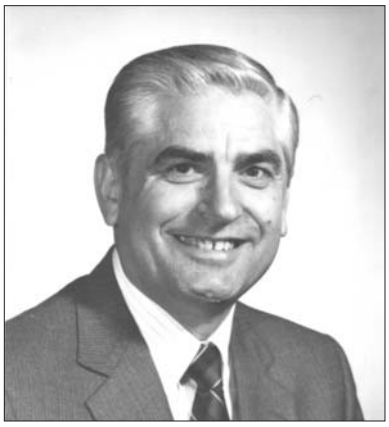

About the Author

Professor George N. Agrios was born in Galarinos, Halkidiki, Greece. He received his B.S. degree in horticulture from the Aristotelian University of Thessaloniki, Greece, in 1957, and his Ph.D. degree in plant pathology from Iowa State University in 1960. Following graduation he served 2 years as an officer in the Engineering Corps of the Greek army. In January 1963 he was hired as an assistant professor of plant pathology at the University of Massachusetts at Amherst. His assignment was 50% teaching and 50% research on viral diseases of fruits and vegetables. His teaching included courses in introductory plant pathology, general plant pathology, plant virology, and diseases of florist's crops. His research included studies on epidemiology, genetics, and physiology of viral diseases of apple, cucurbits, pepper, and corn, in which he directed the studies of 25 graduate students and published numerous journal publications. Dr. Agrios was promoted to associate professor in 1969 and to professor in 1976.

In 1969, he published the first edition of the textbook "Plant Pathology" through Academic Press. The book was adopted for plant pathology classes at almost all universities of the United States and Canada and of most other English-speaking countries. The first edition was later followed by the 2nd edition (1978), 3rd edition (1987), and 4th edition (1997). The book was translated into several major languages, including Spanish, Arabic, Chinese, Korean, and Indochinese, and became the standard plant pathology text throughout the world.

In the meantime, Dr. Agrios served on several departmental, college and university committees as well as committees of the northeastern division of the American Phytopathological Society (APS) and of the national APS. He was elected president of the northeastern division (1980) of APS. He was instrumental in founding the APS Press, of which he served as the first editor-in-chief (1984–1987). He was elected vice-president of APS in 1988, serving as vice-president , president-elect, and president (1990 and 1991). In 1988, professor Agrios accepted a position as chairman of the Plant Pathology Department of the University of Florida, overseeing approximately 50 Ph.D. plant pathologist faculty. Half of the faculty were located at the university campus in Gainesville, Florida, while the others worked at 1 of 13 agricultural research centers throughout the state of Florida where they studied all types of diseases of various crops. In 1999, the Florida Board of Regents approved the establishment of the new and unique Doctor of Plant Medicine Program and professor Agrios was appointed its first director. In 2002, Dr. Agrios relinquished his position as chairman of the Plant Pathology Department to concentrate on his duties as director of the Doctor of Plant Medicine Program. In June 2002, however, health reasons forced Dr. Agrios to retire from the University of Florida.

GENERAL ASPECTS

chapter one

INTRODUCTION

PROLOGUE: THE ISSUES

Plant pathology is a science that studies plant diseases and attempts to improve the chances for survival of plants when they are faced with unfavorable environmental conditions and parasitic microorganisms that cause disease. As such, plant pathology is challenging, interesting, important, and worth studying in its own right. It is also, however, a science that has a practical and noble goal of protecting the food available for humans and animals. Plant diseases, by their presence, prevent the cultivation and growth of food plants in some areas; or food plants may be cultivated and grown but plant diseases may attack them, destroy parts or all of the plants, and reduce much of their produce, i.e., food, before they can be harvested or consumed. In the pursuit of its goal, plant pathology is joined by the sciences of entomology and weed science.

It is conservatively estimated that diseases, insects, and weeds together annually interfere with the production of, or destroy, between 31 and 42% of all crops produced worldwide (Table 1-1). The losses are usually lower in the more developed countries and higher in the developing countries, i.e., countries that need food the most. It has been estimated that of the 36.5% average of total losses, 14.1% are caused by diseases, 10.2% by insects, and 12.2% by weeds.

Considering that 14.1% of the crops are lost to plant diseases alone, the total annual worldwide crop loss from plant diseases is about $220 billion. To these should be added 6–12% losses of crops after harvest, which are particularly high in developing tropical countries where training and resources such as refrigeration are generally lacking. Also, these losses do not include losses caused by environmental factors such as freezes, droughts, air pollutants, nutrient deficiencies, and toxicities.

Although impressive, the aforementioned numbers do not tell the innumerable stories of large populations in many poor countries suffering from malnutrition, hunger, and starvation caused by plant diseases; or of lost income and lost jobs resulting from crops destroyed by plant diseases, forcing people to leave their farms and villages to go to overcrowded cities in search of jobs that would help them survive.

Moreover, the need for measures to control plant diseases limits the amount of land available for cultivation each year, limits the kinds of crops that can be grown in fields already contaminated with certain microorganisms, and annually necessitates the use of millions of kilograms of pesticides for treating seeds, fumigating soils, spraying plants, or the postharvest treatment of fruits. Such control measures not only add to the cost of food production, some of them, e.g., crop rotation, necessarily limit the amount of food that can be produced, whereas others add toxic chemicals to the environment. It is therefore the duty and goal of plant pathology to balance all the factors involved so that the maximum amount of food can be produced with the fewest adverse side effects on the people and the environment.

TABLE 1-1
Estimated Annual Crop Losses Worldwide

Attainable crop production (2002 prices)	$1.5 trillion
Actual crop production (–36.5%)	$950 billion
Production without crop protection	$455 billion
Losses prevented by crop protection	$415 billion
Actual annual losses to world crop production	$550 billion
Losses caused by diseases only (14.1%)	$220 billion

PLANTS AND DISEASE

Plants make up the majority of the earth's living environment as trees, grass, flowers, and so on. Directly or indirectly, plants also make up all the food on which humans and all animals depend. Even the meat, milk, and eggs that we and other carnivores eat come from animals that themselves depend on plants for their food. Plants are the only higher organisms that can convert the energy of sunlight into stored, usable chemical energy in carbohydrates, proteins, and fats. All animals, including humans, depend on these plant substances for survival.

Plants, whether cultivated or wild, grow and produce well as long as the soil provides them with sufficient nutrients and moisture, sufficient light reaches their leaves, and the temperature remains within a certain "normal" range. Plants, however, also get sick. Sick plants grow and produce poorly, they exhibit various types of symptoms, and, often, parts of plants or whole plants die. It is not known whether diseased plants feel pain or discomfort.

The agents that cause disease in plants are the same or very similar to those causing disease in humans and animals. They include pathogenic microorganisms, such as viruses, bacteria, fungi, protozoa, and nematodes, and unfavorable environmental conditions, such as lack or excess of nutrients, moisture, and light, and the presence of toxic chemicals in air or soil. Plants also suffer from competition with other, unwanted plants (weeds), and, of course, they are often damaged by attacks of insects. Plant damage caused by insects, humans, or other animals is not usually included in the study of plant pathology.

Plant pathology is the study of the organisms and of the environmental factors that cause disease in plants; of the mechanisms by which these factors induce disease in plants; and of the methods of preventing or controlling disease and reducing the damage it causes. Plant pathology is for plants largely what medicine is for humans and veterinary medicine is for animals. Each discipline studies the causes, mechanisms, and control of diseases affecting the organisms with which it deals, i.e., plants, humans, and animals, respectively.

Plant pathology is an integrative science and profession that uses and combines the basic knowledge of botany, mycology, bacteriology, virology, nematology, plant anatomy, plant physiology, genetics, molecular biology and genetic engineering, biochemistry, horticulture, agronomy, tissue culture, soil science, forestry, chemistry, physics, meteorology, and many other branches of science. Plant pathology profits from advances in any one of these sciences, and many advances in other sciences have been made in attempts to solve plant pathological problems.

As a science, plant pathology tries to increase our knowledge about plant diseases. At the same time, plant pathology tries to develop methods, equipment, and materials through which plant diseases can be avoided or controlled. Uncontrolled plant diseases may result in less food and higher food prices or in food of poor quality. Diseased plant produce may sometimes be poisonous and unfit for consumption. Some plant diseases may wipe out entire plant species and many affect the beauty and landscape of our environment. Controlling plant disease results in more food of better quality and a more aesthetically pleasing environment, but consumers must pay for costs of materials, equipment, and labor used to control plant diseases and, sometimes, for other less evident costs such as contamination of the environment.

In the last 100 years, the control of plant diseases and other plant pests has depended increasingly on the extensive use of toxic chemicals (pesticides). Controlling plant diseases often necessitates the application of such toxic chemicals not only on plants and plant products that we consume, but also into the soil, where many pathogenic microorganisms live and attack the plant roots. Many of these chemicals have been shown to be toxic to nontarget microorganisms and animals and may be toxic to humans. The short- and long-term costs of environmental contamination on human health and welfare caused by our efforts to control plant diseases (and other pests) are difficult to estimate. Much of modern research in plant pathology aims at finding other environmentally friendly means of controlling plant diseases. The most promising approaches in-clude conventional breeding and genetic engineering of disease-resistant plants, application of disease-suppressing cultural practices, RNA- and gene-silencing techniques, of plant defense-promoting, nontoxic substances, and, to some extent, use of biological agents antagonistic to the microorganisms that cause plant disease.

The challenges for plant pathology are to reduce food losses while improving food quality and, at the same time, safeguarding our environment. As the world population continues to increase while arable land and most other natural resources continue to decrease, and as our environment becomes further congested and stressed, the need for controlling plant diseases effectively and safely will become one of the most basic necessities for feeding the hungry billions of our increasingly overpopulated world.

The Concept of Disease in Plants

Because it is not known whether plants feel pain or discomfort and because, in any case, plants do not speak or otherwise communicate with us, it is difficult to pinpoint exactly when a plant is diseased. It is accepted that a plant is healthy, or normal, when it can carry out its physiological functions to the best of its genetic potential. The meristematic (cambium) cells of a healthy plant divide and differentiate as needed, and different types of specialized cells absorb water and nutrients from the soil; translocate these to all plant parts; carry on photosynthesis, translocate, metabolize, or store the photosynthetic products; and produce seed or other reproductive organs for survival and multiplication. When the ability of the cells of a plant or plant part to carry out one or more of these essential functions is interfered with by either a pathogenic organism or an adverse environmental factor, the activities of the cells are disrupted, altered, or inhibited, the cells malfunction or die, and the plant becomes diseased. At first, the affliction is localized to one or a few cells and is invisible. Soon, however, the reaction becomes more widespread and affected plant parts develop changes visible to the naked eye. These visible changes are the symptoms of the disease. The visible or otherwise measurable adverse changes in a plant, produced in reaction to infection by an organism or to an unfavorable environmental factor, are a measure of the amount of disease in the plant. Disease in plants, then, can be defined as the series of invisible and visible responses of plant cells and tissues to a pathogenic organism or environmental factor that result in adverse changes in the form, function, or integrity of the plant and may lead to partial impairment or death of plant parts or of the entire plant.

The kinds of cells and tissues that become affected determine the type of physiological function that will be

disrupted first (Fig. 1-1). For example, infection of roots may cause roots to rot and make them unable to absorb water and nutrients from the soil; infection of xylem vessels, as happens in vascular wilts and in some cankers, interferes with the translocation of water and minerals to the crown of the plant; infection of the foliage, as happens in leaf spots, blights, rusts, mildews, mosaics, and so on, interferes with photosynthesis; infection of phloem cells in the veins of leaves and in the bark of stems and shoots, as happens in cankers and in diseases caused by viruses, mollicutes, and protozoa, interferes with the downward translocation of photosynthetic products; and infection of flowers and fruits interferes with reproduction. Although infected cells in most diseases are weakened or die, in some diseases, e.g., in crown gall, infected cells are induced to divide much faster (hyperplasia) or to enlarge a great deal more (hypertrophy) than normal cells and to produce

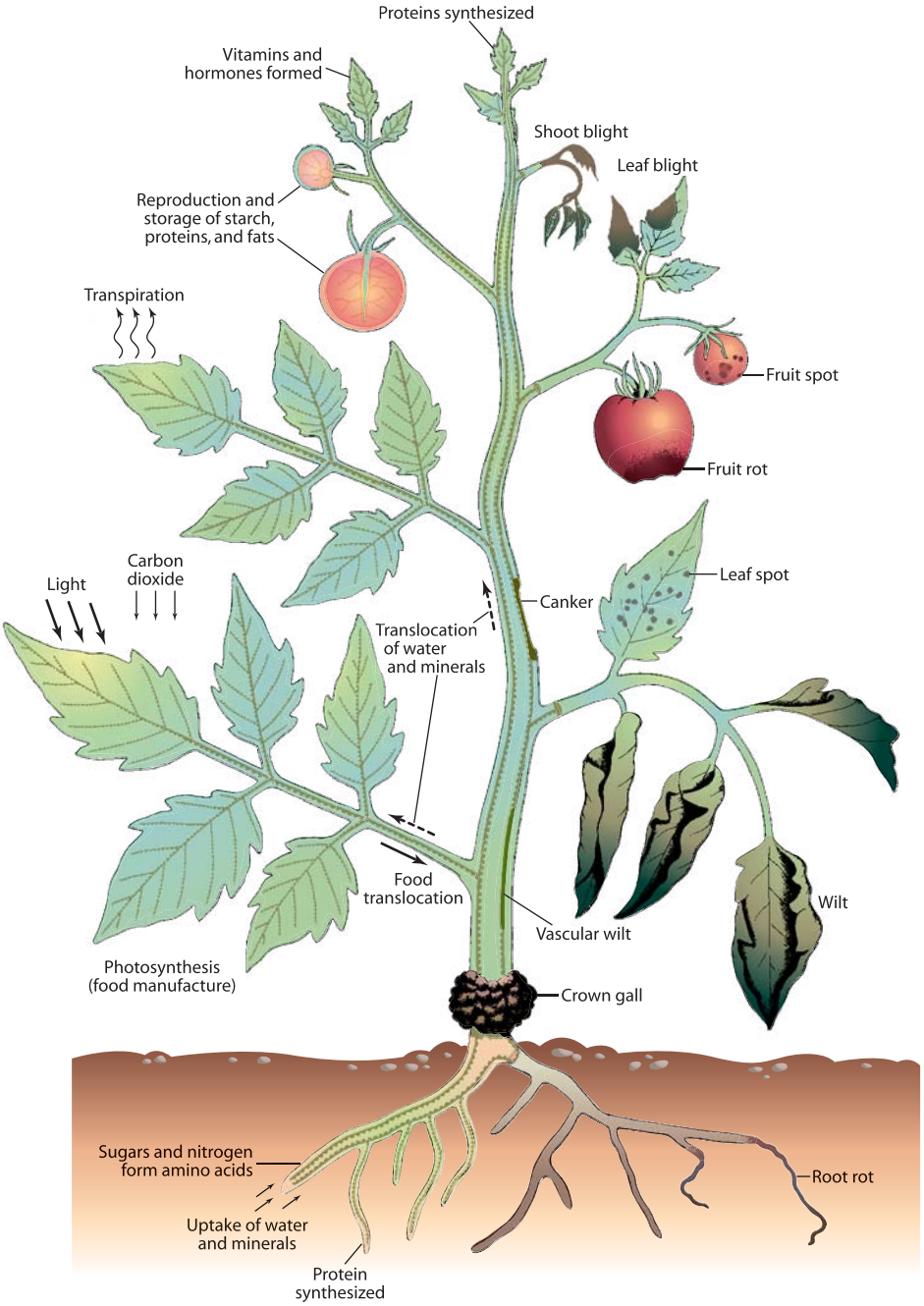

FIGURE 1-1 Schematic representation of the basic functions in a plant (left) and of the kinds of interference with these functions (right) caused by some common types of plant diseases.

abnormal amorphous overgrowths (tumors) or abnormal organs.

Pathogenic microorganisms, i.e., the transmissible biotic (= living) agents that can cause disease and are generally referred to as pathogens, usually cause disease in plants by disturbing the metabolism of plant cells through enzymes, toxins, growth regulators, and other substances they secrete and by absorbing foodstuffs from the host cells for their own use. Some pathogens may also cause disease by growing and multiplying in the xylem or phloem vessels of plants, thereby blocking the upward transportation of water or the downward movement of sugars, respectively, through these tissues. Environmental factors cause disease in plants when abiotic factors, such as temperature, moisture, mineral nutrients, and pollutants, occur at levels above or below a certain range tolerated by the plants.

Types of Plant Diseases

Tens of thousands of diseases affect cultivated and wild plants. On average, each kind of crop plant can be affected by a hundred or more plant diseases. Some pathogens affect only one variety of a plant. Other pathogens affect several dozen or even hundreds of species of plants. Plant diseases are sometimes grouped according to the symptoms they cause (root rots, wilts, leaf spots, blights, rusts, smuts), to the plant organ they affect (root diseases, stem diseases, foliage diseases), or to the types of plants affected (field crop diseases, vegetable diseases, turf diseases, etc.). One useful criterion for grouping diseases is the type of pathogen that causes the disease (see Figs. 1-2 and 1-3). The advantage of such a grouping is that it indicates the cause of the disease, which immediately suggests the probable

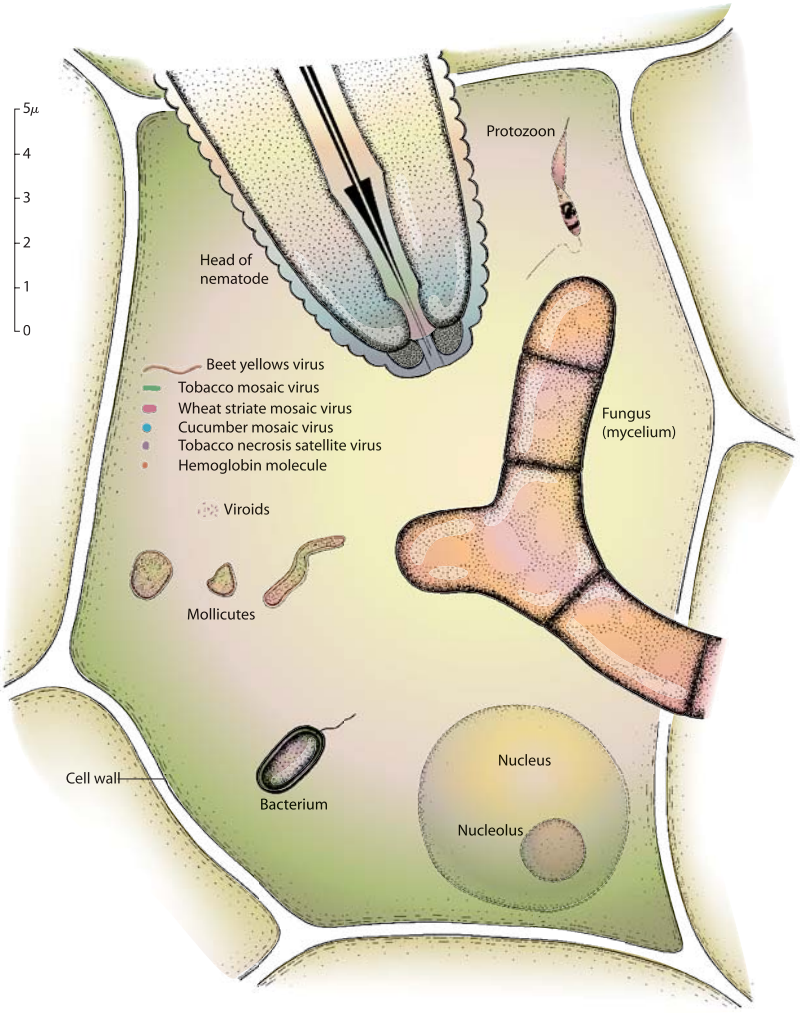

FIGURE 1-2 Schematic diagram of the shapes and sizes of certain plant pathogens in relation to a plant cell. Bacteria, mollicutes, and protozoa are not found in nucleated living plant cells.

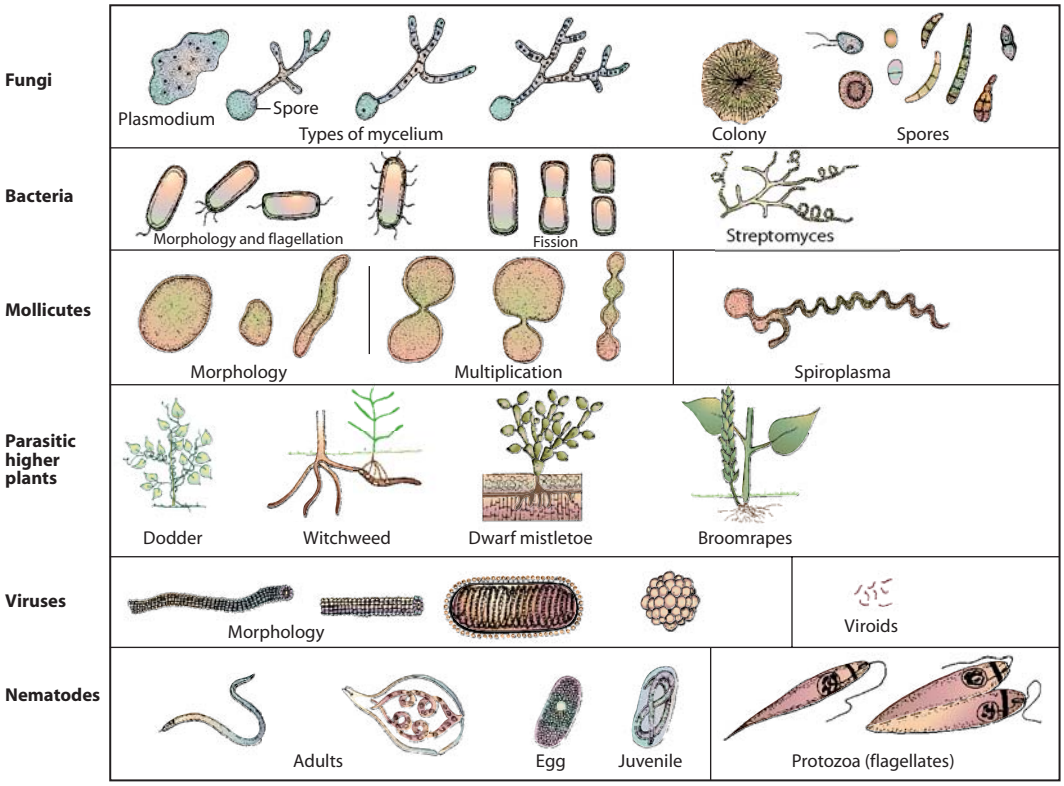

FIGURE 1-3 Morphology and ways of multiplication of some of the groups of plant pathogens.

development and spread of the disease and also possible control measures. On this basis, plant diseases in this text are classified as follows:

I. Infectious, or biotic, plant diseases
   1. Diseases caused by fungi (Figs. 1-4A and 1-4B)
   2. Diseases caused by prokaryotes (bacteria and mollicutes) (Figs. 1-4C and 1-4D)
   3. Diseases caused by parasitic higher plants (Fig. 1-5A) and green algae
   4. Diseases caused by viruses and viroids (Fig. 1-5B)
   5. Diseases caused by nematodes (Fig. 1-5C)
   6. Diseases caused by protozoa (Fig. 1-5D)

II. Noninfectious, or abiotic, plant diseases (Fig. 10-1)
   1. Diseases caused by too low or too high a temperature
   2. Diseases caused by lack or excess of soil moisture
   3. Diseases caused by lack or excess of light
   4. Diseases caused by lack of oxygen
   5. Diseases caused by air pollution
   6. Diseases caused by nutrient deficiencies
   7. Diseases caused by mineral toxicities
   8. Diseases caused by soil acidity or alkalinity (pH)
   9. Diseases caused by toxicity of pesticides
   10. Diseases caused by improper cultural practices

HISTORY OF PLANT PATHOLOGY AND EARLY SIGNIFICANT PLANT DISEASES

Introduction

Even when humans lived as hunters or nomads and their food consisted only of meat or leaves, fruit, and seeds, which they picked wherever they could find them, plant diseases took their toll on hunted animals and on humans. Plant diseases caused leaves and shoots to mildew and blight, and fruit and seeds to rot, thereby forcing humans to keep looking until they could find enough healthy fruit or food plants of some kind to satisfy their hunger. As humans settled down and became farmers, they began growing one or a few kinds of food plants in small plots of land and depended on these plants for their survival throughout the year. It is probable that every year, and in some years more than in others, part of the crop was lost to diseases. In such years food supplies were insufficient and hunger was common. In years when wet weather favored the development of plant diseases, most or all of the crop was

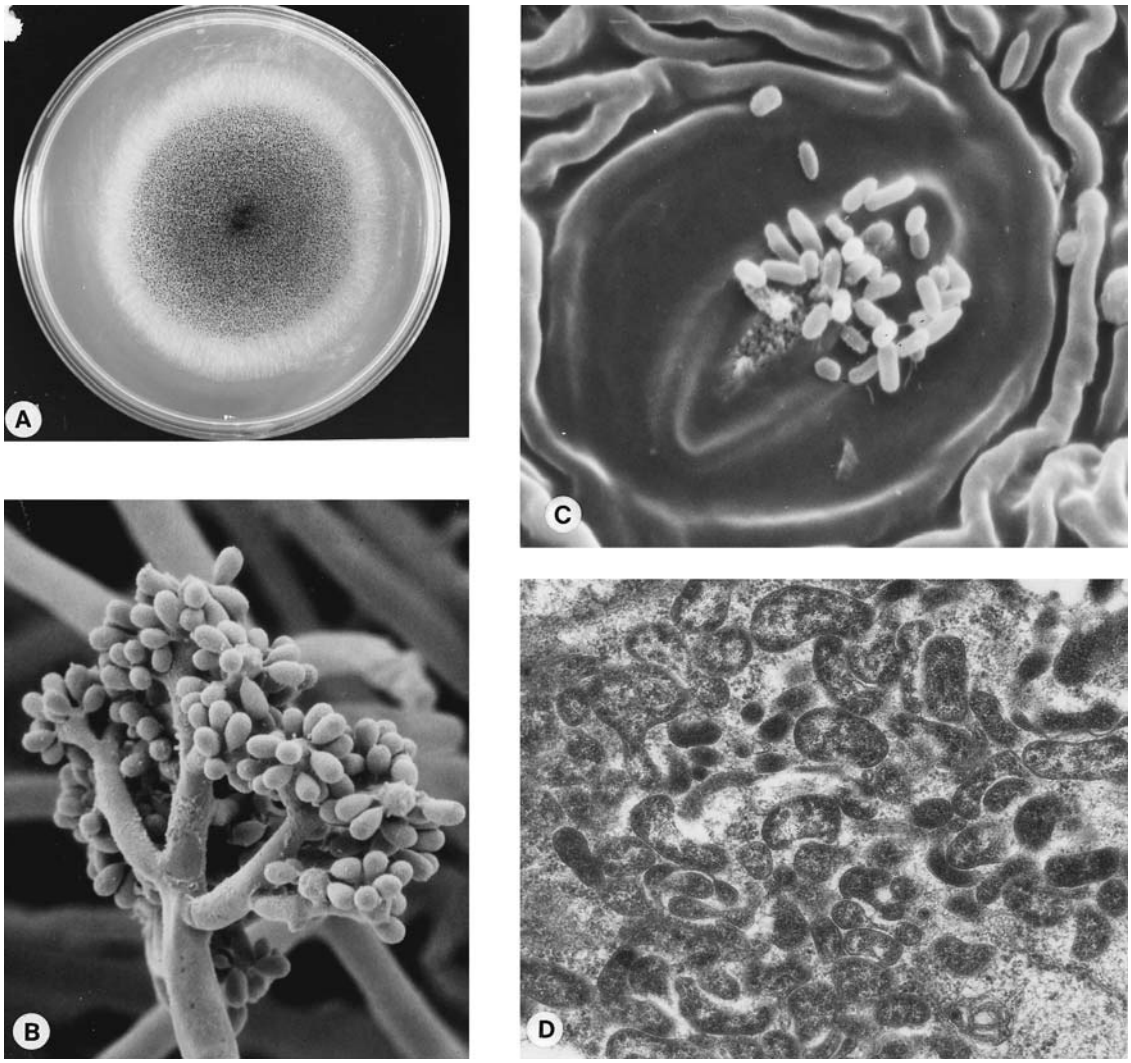

FIGURE 1-4 Three types of pathogenic microorganisms that cause plant diseases. (A) Fungus growing out of a piece of infected plant tissue placed in the center of a culture plate containing nutrient medium. (B) Mycelium and spores of a plant pathogenic fungus (*Botrytis* sp.) (600×). (C) Bacteria at a stoma of a plant leaf (2500×). (D) Phytoplasmas in a phloem cell of a plant (5000×). [Photographs courtesy of (B) M. F. Brown and H. G. Brotzman, (C) L. Mansvelt, I. M. M. Roos, and M. J. Hattingh, and (D) J. W. Worley.]

destroyed and famines resulted, causing immense suffering and probably the death of many humans and animals from starvation. It is not surprising, therefore, that plant diseases are mentioned in some of the oldest books available (Homer, c. 1000 B.C., Old Testament, c. 750 B.C.) and were feared as much as human diseases and war.

BOX 1 Plant diseases as the wrath of gods — theophrastus

The climate and soil of countries around the eastern Mediterranean Sea, from where many of the first records of antiquity came to us, allow the growth and cultivation of many plants. The most important crop plants for the survival of people and of domesticated animals were seed-producing cereals, especially wheat, barley, rye, and oats; and legumes, especially beans, fava beans, chickpeas, and lentils. Fruit trees such as apple, citrus, olives, peaches, and figs, as well as grapes, melons, and squash, were also cultivated. All of these crop plants suffered losses annually due to drought, insects, diseases, and weeds. Because most families grew their own crops and depended on their produce for survival

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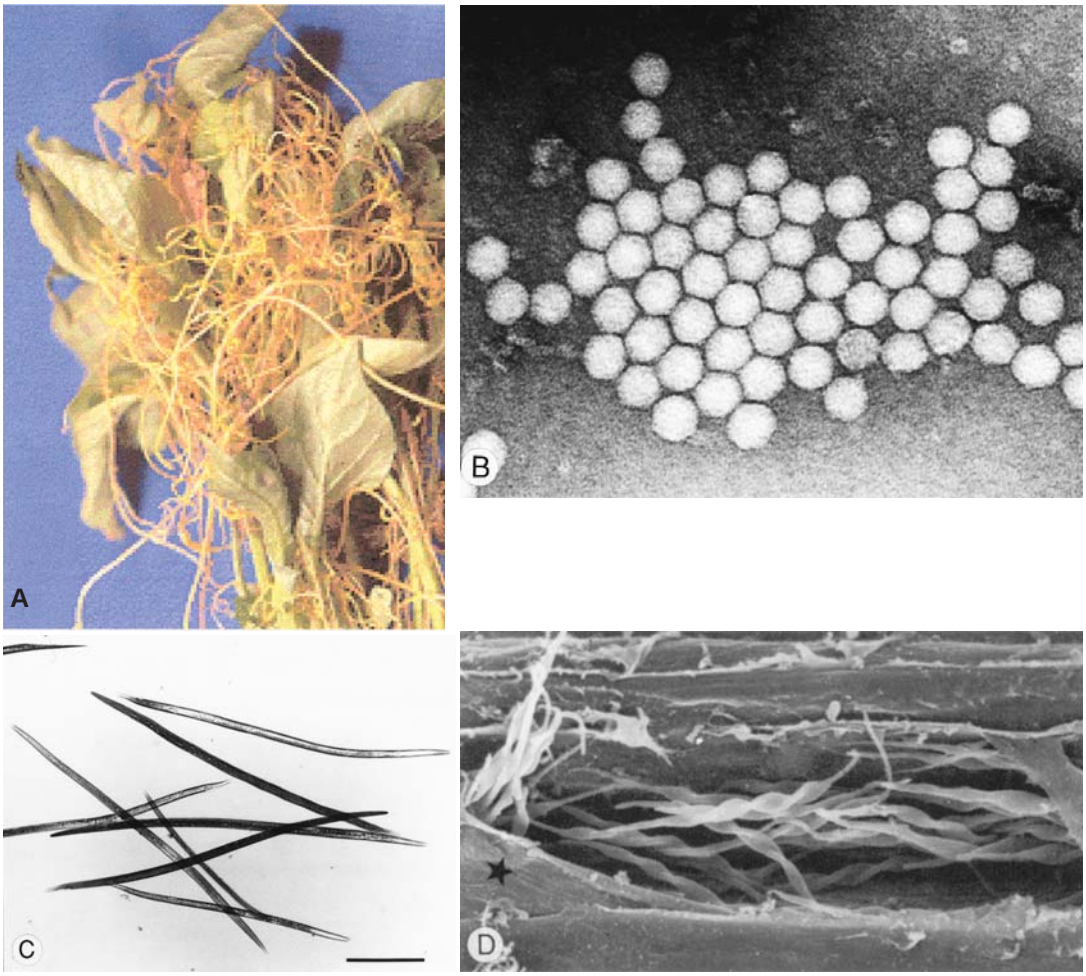

FIGURE 1-5 The other four types of pathogens that cause plant disease. (A) Thread-like parasitic higher plant dodder (*Cuscuta* sp.) parasitizing pepper seedlings. (B) *Tobacco ringspot virus* isolated from infected tobacco plants (200,000×). (C) Plant parasitic nematodes (*Ditylenchus* sp.) isolated from infected onion bulbs (80×). (D) Protozoa (*Phytomonas* spp.) in a phloem cell of an oil palm root (4000×) [Photographs courtesy of (A) G. W. Simone, (C) N. Greco, supplied courtesy R. Inserra, and (D) W. de Sousa].

until the next crop was produced the following year, losses of any amount of crops, regardless of cause, created serious hunger and survival problems for them. Occurrences of mildews (Fig. 1-6, see also pages 448–452), blasts (Figs. 1-7 A, 1-7B, 1-8A and 1-8B, see also pages 582–591), and blights on cereals (Figs. 1-9 A and 1-9B, see also pages 562–571) and legumes (Figs. 1-10A and 1-10B) are mentioned in numerous passages of books of the Old Testament (about 750 B.C.) of the Bible. Blasts, probably the smut diseases, destroyed some or all kernels in a head by replacing them with fungal spores. Blights, probably rusts, weakened the plants and used up the nutrients and water that would fill the kernels, leaving the kernels shriveled and empty (Fig. 1-9B).

Mention of plant diseases is found again in the writings of the Greek philosopher Democritus, who, around 470 B.C., noted plant blights and described a way to control them. It was not, however, until another Greek philosopher, Theophrastus (Fig. 1-11, c. 300 B.C.) made plants and, to a much smaller extent, plant diseases the object of a systematic study. Theophrastus was a pupil of Aristotle and later became his successor in the school. Among others, Theophrastus wrote two books on plants. One, called "*The Nature of Plants*," included chapters on the morphology and anatomy of plants and

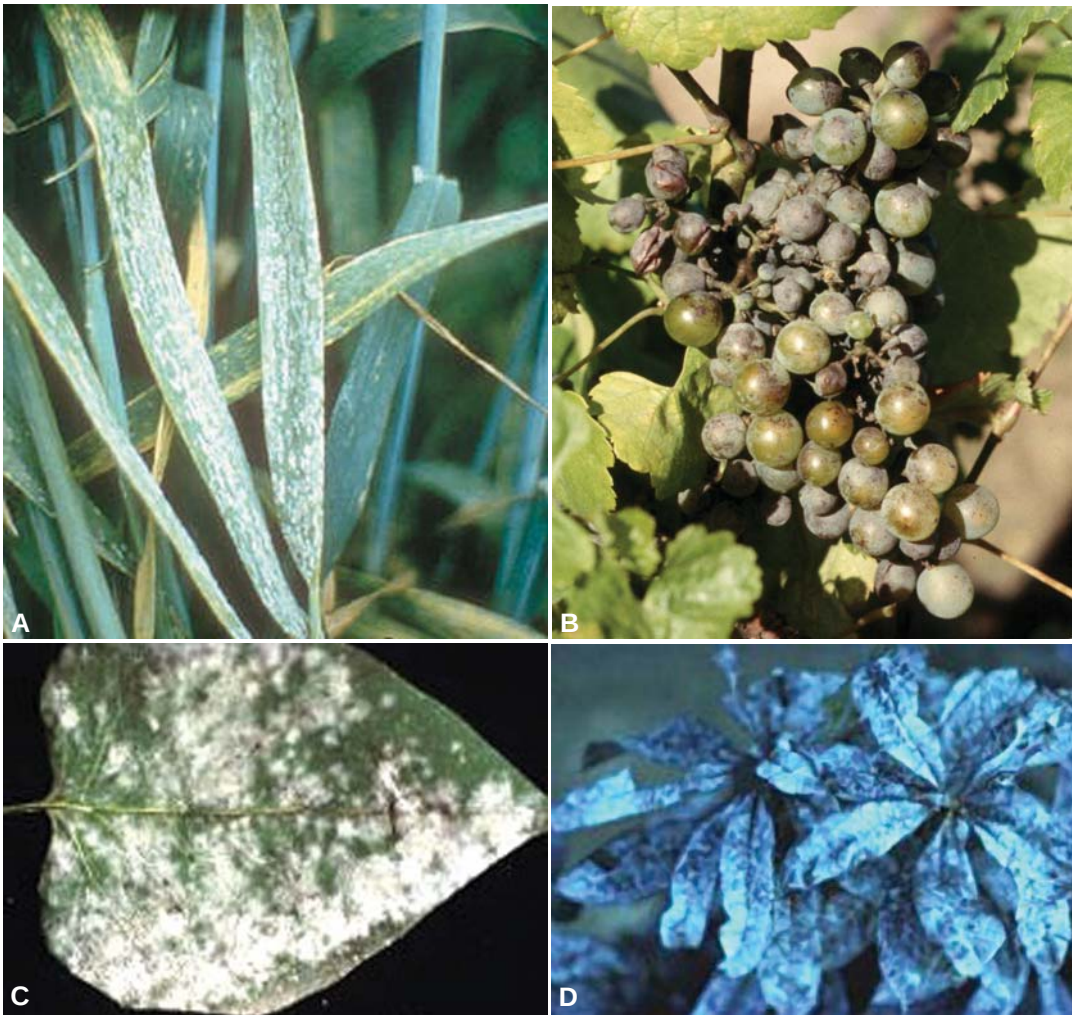

FIGURE 1-6 Powdery mildew symptoms on (A) leaves of young wheat plant, (B) cluster of grape berries, (C) lilac leaf, and (D) azalea plant. [Photographs courtesy of (A) G. Munkvold, Iowa State University, (B) E. Hellman, Texas A&M University, and (C and D) S. Nameth, Ohio State University.]

descriptions of wild and cultivated woody plants, perennial herbaceous plants, wild and cultivated vegetable plants, the cereals, which also included legumes, and medicinal plants and their saps. The other book, called "*Reasons of Vegetable Growth*," included chapters on plant propagation from seeds and by grafting, the environmental changes and their effect on plants, cultural practices and their effect on plants, the origin and propagation of cereals, unnatural influences, including diseases and death of plants, and about the odor and the taste of plants. For these works, Theophrastus has been considered the "*father of botany*."

The contributions of Theophrastus to the knowledge about plant diseases are quite limited and influenced by the beliefs of his times. He observed that plant diseases were much more common and severe in lowlands than on hillsides and that some diseases, e.g., rusts, were much more common and severe on cereals than on legumes. In many of the early references, plant diseases were considered to be a curse and a punishment of the people by God for wrongs and sins they had committed. This implied that plant diseases could be avoided if the people would abstain from sin. Nobody, of course, thought that farmers in the lowlands sinned more than those on the hillsides, yet Theophrastus and his contemporaries, being unable to explain plant diseases, believed that God controlled the weather that "brought about" the disease. They believed that

continued

FIGURE 1-7 Loose smut (blast) of (A) barley and (B) wheat caused by the fungus *Ustilago* sp. [Photographs courtesy of (A) P. Thomas and (B) I. Evans, WCPD.]

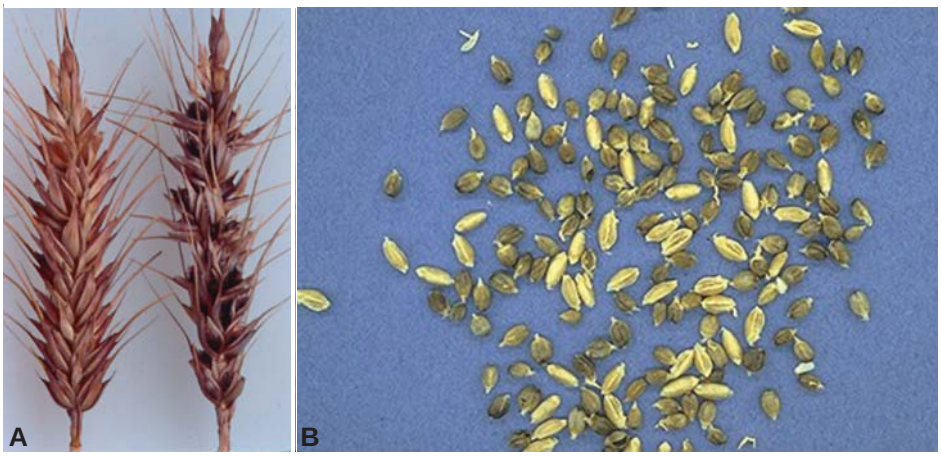

FIGURE 1-8 Cover smut or bunt (blast) of wheat caused by the fungus *Tilletia*. (A) Plant on the left is healthy; plant on the right shows infected, smaller, rounded, black wheat kernels in glumes spread out. (B) Healthy (light colored) and covered smut-infected (dark colored) kernels of wheat. [Photographs courtesy of (A) WCCPD and (B) P. Lipps, Ohio State University.]

FIGURE 1-9 (A) Wheat stems and leaves infected heavily with stem rust of wheat caused by the fungus *Puccinia tritici*. (B) Wheat kernels from rust-infected plants on the left are thin and almost empty of nutrients compared to kernels on the right from a healthy wheat plant, which are plump, full of starch and other nutrients. [Photographs courtesy of (A) CIMMYT and (B) USDA, Cereal Dis. Lab., St. Paul, MN.]

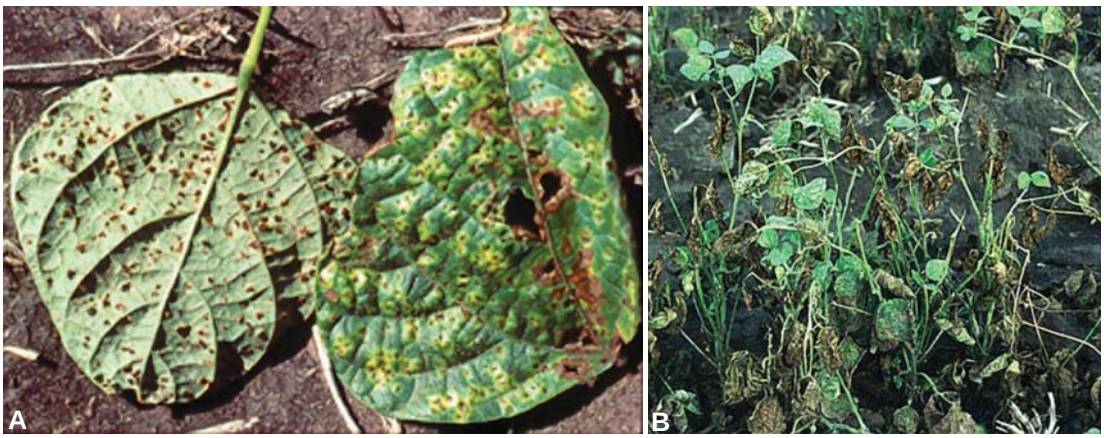

FIGURE 1-10 Close-up of bean rust caused by the fungus *Uromyces appendiculatus*. (A) Rust spots on the upper and lower sides of bean leaves. (B) Rust-infected bean plants in the field showing many leaves killed by the rust and fallen off. [Photographs courtesy of (A) R. G. Platford, WCPD, and (B) J. R. Steadman, University of Nebraska.]

continued

FIGURE 1-11 Theophrastus, the "father of botany."

plant diseases were a manifestation of the wrath of God and, therefore, that avoidance or control of the disease depended on people doing things that would please that same superpower. In the fourth century B.C.; the Romans suffered so much from hunger caused by the repeated destruction of cereal crops by rusts and other diseases that they created a separate god, whom they named Robigus. To please Robigus, the Romans offered prayers and sacrifices in the belief that he would protect them from the dreaded rusts. The Romans even established a special holiday for Robigus, the Robigalia, during which they sacrificed red dogs, foxes, and cows in an attempt to please and pacify Robigus so he would not send the rusts to destroy their crops.

Efforts to control plant diseases were similarly hampered by the lack of information on the causes of disease and by the belief that diseases were manifestations of the wrath of God. Nevertheless, some ancient writers, e.g., Homer (c. 1000 B.C.), mention the therapeutic properties of sulfur on plant diseases, and Democritus (c. 470 B.C.) recommended controlling plant blights by sprinkling plants with the olive grounds left after extraction of the olive oil. Most ancient reports, however, dealt with festivals and sacrifices to thank, please, or appease a god and to keep the god from sending the dreaded rusts, mildews, blasts, or other crop scourges. Very little information on controlling plant diseases was written anywhere for almost 2000 years.

During the two millennia of fatalism, a few important observations were made on the causes and control of plant diseases, but they were not believed by their contemporaries and were completely ignored by the generations that followed. It was not until about A.D. 1200 that a higher plant, the mistletoe, was proposed as a parasite that obtains its food from the host plant, which it makes sick. It was also noted that the host plant can be cured by pruning out the part carrying the mistletoe. Nobody, however, followed up on this important observation.

BOX 2 Mistletoe recognized as the first plant pathogen

Mistletoes are plants that live as parasites on branches of trees (see pages 715) but, for various reasons, they have caught the fancy of people in various cultures and have made a name for themselves way beyond their real properties.

Although mistletoe is the first plant pathogen to be recognized as such and the first pathogen for which a cultural control (by pruning affected branches) was recommended, both by Albertus Magnus (Fig. 1-12A) around 1200 A.D., a great deal more has been fantasized, said, written, and practiced about it than its importance as a pathogen would indicate. Mistletoe, to be sure, both the common or leafy mistletoe (*Viscum* in Europe and elsewhere, *Phoradendron* in North America), which infects many deciduous trees (Figs. 1-12B and 1-12C) and especially the dwarf mistletoes (*Arceuthobium*), which infects conifers, cause considerable damage to trees they infect. In many cases, the evergreen mistletoe plants can be seen clearly after normal leaf fall in the autumn and make up as much as half of the top of the deciduous tree they infect. They generally damage trees by making their trunks and branches swell where they are infected and then break there during windstorms, thereby reducing the surface of the tree and reducing the quality of timber.

Mistletoes, of course, are evergreen parasitic plants that sink their "roots," usually called sinkers or haustoria, into branches of trees. Through the sinkers they absorb all the water and mineral nutrients and most of the organic substances they need from the plant. True

FIGURE 1-12 (A) Albertus Magnus, who recognized the mistletoe as a plant parasite. (B) Tufts of individual mistletoe plants growing on branches of an oak tree in winter. (C) Close-up of a mistletoe plant whose main stems are growing out of the trunk of an oak tree.

mistletoes, however, have well-developed leaves and chlorophyll and carry on photosynthesis and manufacture at least some of the sugars and other organic substances they need. Mistletoe plants produce separate male and female flowers and berry-like fruits containing a single seed. The seeds are coated with a sticky substance and are either forcibly expelled and stick to branches of nearby trees or are eaten by birds but go through their digestive tract and stick to branches on which birds drop them.

The striking visibility of true mistletoes on deciduous trees, and their ability to remain green while their host leaves fall for the winter, excited the imagination of people since the times of the ancient Greeks and inspired many myths and traditions involving the mistletoe plant through the centuries. The plant itself was thought to possess mystical powers and became associated with many folklore customs in many countries. It was thought to bestow life and protect against poison, to act as an aphrodisiac, and to bestow fertility. Mistletoe sprigs placed over house and stable doors or hung from ceilings were believed to ward off witches and evil spirits. The Romans decorated their temples and houses in midwinter with mistletoe to please the gods to whom it was sacred. In Nordic mythology, the mistletoe was sacred to Frigga, the goddess of love, but was used by Loki, the goddess of evil, as an arrow and killed Frigga's son, the god of the summer sun. Frigga managed to revive her son under the mistletoe tree and, in her joy, she kissed everyone who was under the mistletoe tree. But, for its misdeed to her son, she condemned the mistletoe to, be in the future, a parasite and to have no power to cause misfortune, sorrow, or death. She decreed instead that anyone standing under a mistletoe tree was due not only protection from any harm, but also a kiss, a token of peace and love. So, in Scandinavia, mistletoe was thought of as a plant of peace: under the mistletoe, enemies could agree on a truce or feuding spouses could kiss and make up. In England, a ball of mistletoe was decorated with ribbons and ornaments and was hung up at Christmas. If a young lady was standing under the ball, she could not refuse to be kissed or she could not expect to get married the following

continued

year. A couple in love that kiss under the mistletoe is equivalent to promising to marry and a prediction of long life and happiness together. Nowadays, in many parts of Europe and America, a person standing under a ball or even a sprig of mistletoe at Christmastime is inviting to be kissed by members of the opposite gender as a sign of friendship and goodwill. There are, actually, more myths and customs associated with mistletoe. Who would think that a minor parasitic higher plant would excite the imagination of so many others and have so many stories about it.

BOX 3 Plant diseases as the result of spontaneous generation

Following Theophrastus, other than the proposal by Magnus that the mistletoe was a parasite, there was little useful knowledge that was added about plants or about plant diseases for about 2000 years, although there are reports of famines in several parts of the world. Especially bad were outbreaks in northcentral Europe of ergotism, a disease of humans and animals caused from eating grains contaminated with parts of the fungus that causes the ergot disease of cereals (see pages 501–504). People continued to associate plant diseases with sin and the wrath of God and therefore were fatalistic about the occurrence of plant diseases, the repeated losses of food, and the hunger and famines that followed. References to the ravages of plant diseases appeared in the writings of several contemporary historians, but little was added to the knowledge about the causes and control of plant diseases. People everywhere believed that plant diseases, as well as human and animal diseases, just happened spontaneously. Whatever was observed on diseased plants or on diseased plant produce was considered to be the product or the result of the disease rather than the cause of it. After the invention of the compound microscope in the mid-1600s, which enabled scientists to see many of the previously invisible microorganisms, scientists, as well as laypeople, became even stronger believers in the spontaneous generation of diseases and of the microorganisms associated with diseased or decaying plant, human, or animal tissues. That is, they came to believe that the mildews, rusts, decay, or other symptoms observed on diseased plants, and any microorganisms found on or in diseased plant parts, were the natural products of diseases that just happened rather than being the cause and effect of the diseases.

Biology and Plant Pathology in Early Renaissance

People continued to suffer from hunger and malnutrition due partially at least to diseases destroying their crops and their fruit. They, however, continued to consider plant diseases as the work and wish of their God and, therefore, an event that could neither be understood nor avoided. In the mid-1600s, however, a group of French farmers noted that wheat rust was always more severe on wheat near barberry bushes than away from them (Fig. 1-13). The farmers thought that the rust was produced by the barberry plants from which it moved to wheat. They, therefore, asked the French government to pass the first plant disease regulatory legislation that would force towns to cut and destroy the barberry bushes to protect the wheat crop.

FIGURE 1-13 A bush of barberry (*Berberis vulgaris*) growing at the edge of a wheat field and helping close the dioecious disease cycle of wheat stem rust disease. The fungus, *Puccinia graminis*, overwinters on barberry on which it produces spores that infect wheat plants near the barberry (see photo) from which then spores of the fungus spread to more wheat plants. (Photograph courtesy of USDA Cereal Dis. Lab., St. Paul, MN.)

In 1670, the French physician Thoullier observed that ergotism or Holy Fire, a serious and often deadly disease of humans in northcentral Europe (see pages 39 and 559), did not spread from one person to another but seemed to be associated with the consumption of ergot-contaminated grains. At about the same time, Robert Hooke, in England, invented the double-lensed (compound) microscope with which he examined thin slices of cork and called its units "cells." Soon after, the Dutchman Antonius van Leeuwenhoek (Fig. 1-14A) improved significantly the lenses and the structure of the microscope and began to examine not only the anatomy of plants, but also the body of filamentous fungi and algae, protozoa, sperm cells, blood cells, and even bacteria. All of these microorganisms, of course, were considered to be produced by whatever organism (animal

FIGURE 1-14 (A) Antonius van Leeuwenhoek. (B) Carl von Linne'. (C) Charles Darwin.

or plant) or medium they happened to be found in and were not thought of as independent, autonomous organisms. In 1735, the Swedish philosopher–botanist Carl von Linne' (Fig. 1-14B) published his main work "*Systema Naturae*," by which he established the diagnosis of plant species and the binomial nomenclature of plants. Linne's species, however, were rigid and were supposed to have remained unchanged since creation. It was not until more than a century later, in 1859, that the Englishman Charles Darwin (Fig. 1-14C) published his book "*The Origin of Species by Means of Natural Selection*" and showed that species of all organisms, plants and animals, evolve over time and adapt to changes in their environment for survival.

The discovery and availability of the microscope, however, sparked significant interest in microscopic fungi and, subsequently, their possible association with plant diseases. In 1729, the Italian botanist Pier Antonio Micheli described many new genera of fungi and illustrated their reproductive structures. He also noted that when placed on freshly cut slices of melon, these structures grew and produced the same kind of fungus that had produced them. He proposed, therefore, that fungi arise from their own spores rather than spontaneously, but because the "spontaneous generation" theory was so imbedded in people's minds, nobody believed Micheli's evidence. Similarly, in 1743, the English scientist Needham observed nematodes inside small,

abnormally rounded wheat kernels but he, too, failed to show or suggest that they were the cause of the problem.

In 1755, the Frenchman Tillet, working with smutted wheat, showed that he could increase the number of wheat plants developing covered smut (Figs. 1-8A and 1-8B) by dusting wheat kernels before planting with smut dust, i.e., with smut spores (Fig. 1-15). He also noted that he could reduce the number of smutted wheat plants produced by treating the smut-treated kernels with copper sulfate. Tillet, too, however, did not interpret his experiments properly and, instead of concluding that wheat smut is an infectious plant disease, he believed that it was a poisonous substance contained in the smut dust, rather than the living spores and fungus coming from them, that caused the disease. More than 50 years later, in 1807, Prevost, another Frenchman, repeated both the inoculation experiments and those in which the seeds were treated with copper sulfate, as done by Tillet, and he obtained the same results. In addition, Prevost observed smut spores from untreated and treated wheat seed under the microscope and noticed that those from untreated seed germinated and grew whereas those from treated seed failed to germinate. He, therefore, concluded correctly that it was the smut spores that caused the smut disease in wheat and that the reduced number of smutted wheat plants derived from copper sulfate-treated seed was due to the inhibition of germination of smut spores by the copper sulfate. Prevost's conclusions, however, were not accepted by the French Academy of Sciences because its scientists and other scientists throughout Europe still believed that microorganisms and their spores formed through spontaneous generation and were the result rather than the cause of disease. In 1855, a nematode was observed in galls of cucumber roots, but again they were thought to have appeared there spontaneously. These beliefs continued to be held and expounded by scientists until the early 1860s, when, in 1861–1863, Anton deBary (Fig. 1-16A) proved that potato late blight was caused by a fungus and Louis Pasteur (Fig. 1-16B) proved that microorganisms were produced from preexisting microorganisms and that most infectious diseases were caused by germs. The latter established the "*germ theory of disease*," which changed the way of thinking of scientists and led to tremendous progress. Significant

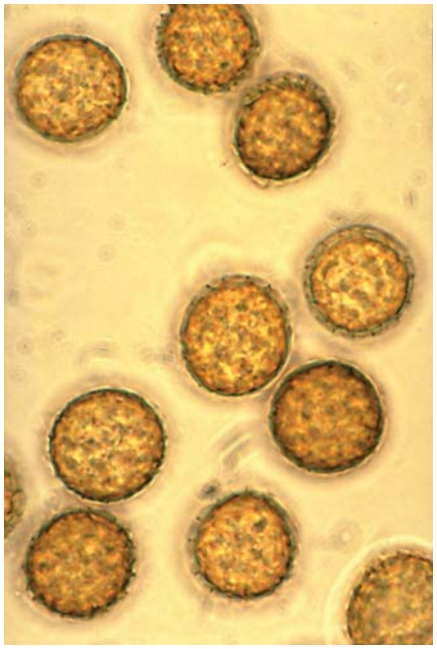

FIGURE 1-15 Teliospores of the fungus *Tilletia*, the cause of the covered smut or bunt of wheat. (Photograph courtesy of M. Babadoost, University of Illinois.)

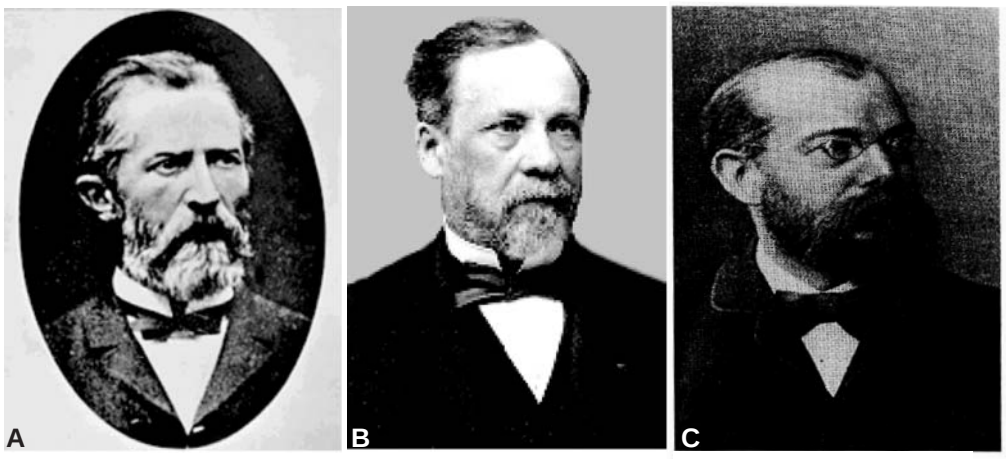

FIGURE 1-16 (A) Anton deBary. (B) Louis Pasteur. (C) Robert Koch.

impetus to this progress was added by Robert Petri, who developed artificial nutrient media for culturing the microorganisms (Petri dishes), and by Robert Koch (Fig. 1-16C), who established that for proving that a certain microorganism was the cause of a particular infectious disease, certain necessary steps (Koch's postulates) must be carried out and certain conditions must be satisfied.

BOX 4 Potato blight and the irish famine: a deadly mix of ignorance and politics

In about 1800, the potato, which was introduced in Europe from South and Central America around 1570 A.D., was a well-established crop in Ireland. After strong objections against adopting it because (1) it was new and not mentioned in the Bible, (2) it was produced in the ground and, therefore, was unclean, and (3) because parts of it were poisonous, the potato was nevertheless adopted and its cultivation spread rapidly. Adoption of potato cultivation came as a result of it producing much more edible food per unit of land than grain crops, mostly wheat and rye, grown until then. It was adopted also because the ground protected it from the pests and diseases that destroyed aboveground crops and from destruction by the soldiers sent by absentee English landlords to collect overdue land rents.

At that time, most Irish farmers were extremely poor, owned no land, and lived in small windowless, one-room huts. The farmers rented land from absentee English landlords who lived in England, and planted grain and other crops. The yields were poor and, in any case, large portions of them had to be used for paying the exorbitant rent so as to avoid eviction. The Irish farmers also kept small plots of land, usually as small as a quarter of an acre and basically survived the winter with the food they produced on that land. Potato production was greatly favored by the cool, wet climate of Ireland, and the farmers began growing and eating potatoes to the exclusion of other crops and foodstuffs. Irish farmers, therefore, became dependent on potatoes for their sustenance and survival. Lacking proper warehouses, the farmers stored their potato tubers for the winter in shallow ditches in the ground. Periodically, they would open up part of the ditch and remove as many potatoes as they thought they would need for the next few weeks.

The potatoes grew well for many years, free of any serious problems. In the early 1840s, potato crops began to fail to varying extents in several areas of Europe and Ireland. Most of the growing season of 1845 in Ireland was quite favorable for the growth of potato plants and for the formation of tubers. Everything looked as though there would be an excellent yield of potatoes everywhere that year. Then, the weather over northern Europe and Ireland became cloudy, wetter, and cooler and stayed that way for several weeks (Fig. 1-17A). The potato crop, which until then looked so promising, began to show blighted leaves and shoots (Fig. 1-17B), and whole potato plants became blighted and died. In just a few weeks, the potato fields in northern Europe and in Ireland became masses of blighted and rotting vegetation (Fig. 1-17C). The farmers were surprised and worried, especially when they noticed that many of the potatoes still in the ground were rotten and others had rotting areas on their surface (Fig. 1-17D). They did what they could to dig up the healthy-looking potatoes from the affected fields and put them in the ditches to hold them through the winter.

The farmer's worry became horror when later in the fall and winter they began opening the ditches and looking for the potatoes they had put in them at harvest. Alas, instead of potatoes they found only masses of rotting tubers (Figs. 1-17D and 1-17E), totally unfit for consumption by humans or animals. The dependence of Irish farmers on potatoes alone meant that they had nothing else to eat — and neither did any of their neighbors. Hunger (Fig. 1-17F) was quickly followed by starvation, which resulted in the death of many Irish. The famine was exacerbated by the political situation between England and Ireland. The British refused to intervene and help the starving Irish with food for several months after the blight destroyed the potatoes. Eventually, by February of the next year (1846), food, in the form of corn from the United States, began to be imported and made available to the starving poor who paid for it by working on various government construction projects. Unfortunately, the weather in 1846 was again cool and wet, favoring the potato blight, which again spread into and destroyed the potato plants and tubers. Hunger, dysentery, and typhus spread among the farmers again, and more of the survivors emigrated to North America. It is estimated that one and a half million Irish died from hunger, and about as many left Ireland, emigrating mostly to the United States of America.

The cause of the destruction of the potato plants and of the rotting of the potato tubers was, of course, unknown and a mystery to all. The farmers and other simple folk believed it to have been brought about by "the little people," by the devil himself whom they tried to exorcise and chase away by sprinkling holy water in the fields, by locomotives traveling the countryside at devilish speeds of up to 20 miles per hour and discharging electricity harmful to crops they went by, or to have been sent by God as punishment for some unspecified sin they had committed. The more educated doctors and clergy were so convinced of the truth of the theory of spontaneous generation that even when they saw the mildewy fungus growth on affected leaves and on some stems and tubers, they thought that this growth was produced by the dying plant as a result of the rotting rather than the cause of the death and rotting of the plant.

Some of the educated people, however, began to have second thoughts about the situation. Dr. J. Lindley, a professor of botany in London, proposed incorrectly that the plants, during the rains, overabsorbed water through their roots and because they could not get rid

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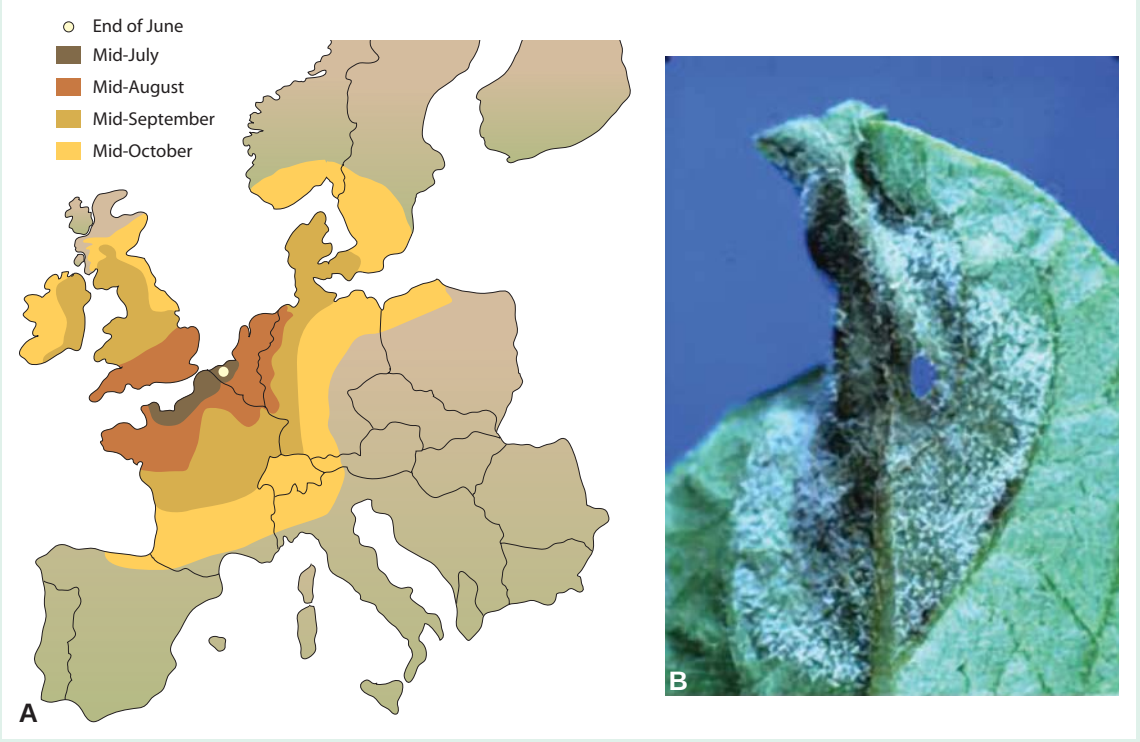

FIGURE 1-17 The late blight of potato and the Irish famine. (A) Itinerary of the advance of the potato blight between June, when the blight was first detected in Belgium, and the end of October 1845, by which time it spread from Italy to Ireland and from Spain to the Scandinavian countries. (B) A young lesion on a potato leaf covered with sporangiophores and sporangiospores of the fungus (oomycete). (C) A potato plant killed completely by the blight (right) next to a healthy-looking resistant plant (left). (D) External and internal appearance of potato tubers infected with the late blight disease. The oomycete is still found near the surface. (E) Advanced invasion and rotting of potato tuber infected with late blight. (F) A period drawing of a family digging for potatoes to avoid starvation during the Irish famine. [Photographs courtesy of (A) W. E. Fry, Cornell University, (B) D. P. Weingartner, University of Florida, (C and D) Cornell University, (E) USDA, and (F) Illustrated London News, 1849.]

of the excess water, their tissues became swollen and rotted. The Reverend Dr. Miles Berkeley, however, noticed that the mold covering potato plants about to rot was a fungus (oomycete) similar but not identical to a fungus he observed on a sick onion. The fungus on potato, however, was identical to a fungus recovered from sick potato plants in northern Europe. Berkeley concluded that this fungus was the cause of the potato blight, but when he proposed it in a letter to a newspaper, it was considered as an incredible and bizarre theory unsupported by facts. The puzzle of what caused blight of potato continued unanswered for 16 years after the 1845 destruction of potatoes by the blight. Finally, in 1861, Anton deBary (Fig. 1-16A) did a simple experiment that proved that the potato blight was caused by a fungus. DeBary simply planted two sets of healthy potatoes, one of which he dusted with spores of the fungus collected from blighted potato plants. When the tubers germinated and began to produce potato plants, the healthy tubers produced healthy plants, whereas the healthy tubers dusted with the spores of the fungus produced plants that became blighted and died. No matter how many times deBary repeated the experiment, only tubers treated with the fungus became infected and produced plants that became infected. Therefore, the fungus, which, we know now, is an oomycete was named *Phytophthora infestans ("infectious plant destroyer"* from phyto = plant, phthora = destruction, infestans = infectious), was the cause of the potato blight. DeBary also showed that the fungus did not just reappear from nowhere the following growing season but instead survived the winter in partially infected potato tubers in the field or storage. In the spring, the fungus infected young plants coming from these partially rotten tubers, produced new spores on these plants, and the spores then spread to other cultivated potato plants that were infected and killed. With this experiment deBary actually disproved the **theory of spontaneous generation**, which stated that microorganisms are produced spontaneously by dying and dead plants and animals, and ushered in the **germ theory of disease**. The honor for this proof, however, is reserved for Louis Pasteur, who proved the theories while working with bacteria at about the same time, 1861–1863, that deBary published his work with the potato blight fungus.

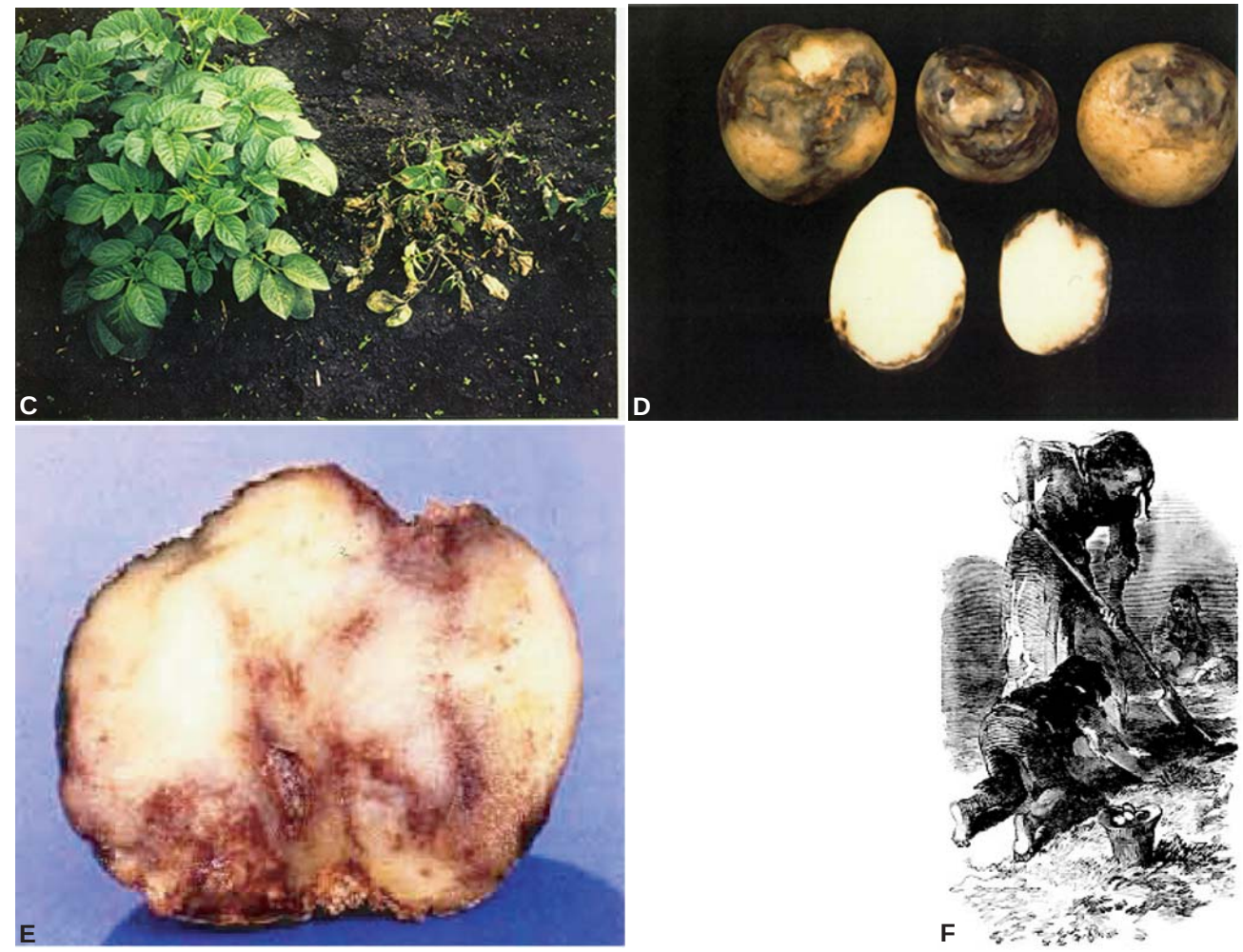

FIGURE 1-17 (*Continued*)

The Expanding Role of Fungi as Causes of Plant Disease

Following the observation by French farmers around the mid-1600s and, independently, by Connecticut farmers in the early 1700s that wheat rust was worse near barberry bushes, the farmers came to believe that barberry fathered the rust, which then moved to wheat. The request by farmers for legislation to force towns to eradicate barberries and in that way to protect the wheat plants from rust followed. At about the same time, spores of the rust fungus were observed with the compound microscope for the first time in England (Hooke, 1667). In Italy, Micheli 60 years later (1729) described many new genera of fungi, illustrated their reproductive structures, and noted that when he placed them on freshly cut slices of melon, these fungal structures generally reproduced the same kind of fungus that produced them. He proposed that fungi arose from their own spores rather than spontaneously, but nobody believed him. New information about plant pathogenic fungi continued to be developed, but most of it was not accepted by the scientists of the time for a long time.

As mentioned previously, in 1755, Tillet in France showed that wheat smut is a contagious plant disease, but even he believed that it was a poisonous substance contained in the smut dust, rather than a living microorganism, that caused the disease. In 1807, Prevost, also in France, repeated and expanded Tillet's experiments and appeared to have demonstrated conclusively that wheat smut was caused by a fungus. His conclusions, however, were not accepted because the scientists were blinded by the belief that microorganisms and their

spores were the result rather than the cause of disease. These beliefs continued to be shared and expounded by scientists for at least another 50 years.

The devastating epidemics of late blight of potato in northern Europe, particularly Ireland, in the 1840s not only dramatized the effect of plant diseases on human suffering and survival, but also greatly stimulated interest in their causes and control. In 1861, deBary finally established experimentally beyond criticism that a fungus (*Ph. infestans*) was the cause of the plant disease known as late blight of potato, a disease that closely resembles the downy mildews.

It is, perhaps, worth noting here that it was during those years (1860–1863) that Louis Pasteur proposed, and finally provided irrefutable evidence, that microorganisms arise only from preexisting microorganisms and that fermentation is a biological phenomenon, not just a chemical one. Pasteur's conclusions, however, were not generally accepted for many years afterward. Nevertheless, the proof for involvement of microorganisms (germs) in fermentation and disease signaled the beginning of the end of the theory of spontaneous generation and provided the basis for the germ theory of disease.

Although fungi had already been the object of study by many scientists, proof that they were causing disease in plants greatly increased interest in them. DeBary himself also carried out studies of the smut and rust fungi, of the fungi causing downy mildews, and of the fungus *Sclerotinia*, which induces rotting of vegetables. The German Kühn in the 1870s and later contributed significantly to the studies of infection and development of smut in wheat plants and promoted the development and application of control measures, particularly seed treatment for cereals. Kühn also wrote the first book on plant pathology, "*Diseases of Cultivated Crops, Their Causes and Their Control*," in which he recognized that plant diseases are caused by an unfavorable environment but can also be caused by parasitic organisms such as insects, fungi, and parasitic plants.

During the years of Pasteur and Koch, several scientists also made significant contributions to plant pathology and to biology and medicine. After establishing beyond criticism in 1861 that the potato blight was caused by a fungus, DeBary went on to show conclusively that smut and rust fungi were also the causes and not the results of their respective plant diseases. Moreover, he showed that some rust diseases require two alternate host plants (see Fig. 1-13) to complete their life cycle, e.g., the fungus causing the stem rust of wheat requires wheat and barberry. DeBary also showed (1886) that some fungi induce rotting of vegetables (Fig. 1-18) by secreting substances (enzymes) that diffuse into plant tissues in advance of the pathogen.

FIGURE 1-18 Infection and advanced internal rotting of summer squash (A) by the fungus *Choanephora*, of peach fruit (B) by the fungus *Rhizopus* sp., and (C) of kiwi fruit by the fungus *Botrytis cinerea*. In all cases, fruit rot is a result of, primarily, pectinolytic enzymes secreted by the fungi and advancing ahead of the mycelium. A small amount of the fungi can be seen on the surface of the fruits. (C) Courtesy of T. Michailides, University of California.

The Discovery of Other Causes of Infectious Diseases

Although Leeuwenhoek first saw microbes with the microscope he invented in 1674, little progress was made toward the concept of microbes as the cause of disease for almost another 200 years. In 1776, Jenner introduced vaccination against the virus-induced smallpox, an extremely infectious and severe disease that used to kill 10 to 20% of those infected, but could only speculate as to its cause and how it worked. In 1861, however, deBary showed that the potato blight was caused by a fungus while Pasteur formulated the germ theory of fermentation. In 1864, Pasteur invented pasteurization and, in 1880, made the first vaccine against the chicken cholera. In the meantime, in 1876, Koch identified the anthrax bacillus, *Bacillus anthracis*, as the first bacterium to cause disease in animals and humans. In addition, in 1887, Koch formulated his rules of disease diagnosis that became known as "Koch's postulates." These rules became the standard procedure for proving that a disease is caused by a bacterium or any other kind of pathogen.

Nematodes

The first report of nematodes associated with a plant disease was made in England by Needham in 1743. He observed nematodes (Fig. 1-19A) within small, abnormally rounded wheat kernels (wheat galls; Fig. 1-19B); however, he did not show or suggest that they were the cause of the disease. It was not until 1855 that a second

FIGURE 1-19 (A) A typical nematode. (B) Wheat seed galls, each filled with as many as 30,000 nematodes. (C) M. Woronin. (D) Clubroot of cabbage caused by the protozoon *Plasmodiophora brassicae*. [Photographs courtesy of (A and B) USDA Nematology Laboratory, Beltsville, Maryland, and (D) C. M. Ocamp, Oregon State University.]

nematode, the root knot nematode, was observed in cucumber root galls. In the next 4 years two other plant parasitic nematodes, the bulb and stem nematode and the sugarbeet cyst nematode, were reported from infected plant parts. Several more nematodes parasitizing plants were described in the early part of the 20th century by Cobb, who made numerous significant contributions to plant nematology.

Protozoan Myxomycetes

In 1878, Woronin (Fig. 1-19C), in Russia, was the first to show that a plant disease, the clubroot disease of cabbage (Fig. 1-19D), was caused by a fungus that has been shown to be a protozoan plasmodiophoromycete. These are fungus-like, single-celled microorganisms that lack a cell wall and, as a result, produce an amoeba-like body called a plasmodium and zoospores. These microorganisms used to be thought of as lower fungi but are now considered members of a different kingdom, the kingdom protozoa.

Bacteria

Soon after Koch showed that bacteria cause disease in animals and humans, Burrill in Illinois showed, in 1878, that bacteria (Fig. 1-20A) caused the fire blight disease (Fig. 1-20B) of pear and apple. Following Burrill's discovery, several other plant diseases were shown, particularly by Erwin Smith (Fig. 1-20C) of the U.S. Department of Agriculture (USDA), to be caused by bacteria. In the early 1890s, Smith was the first to show that crown gall disease (Fig. 1-20D), which he considered similar to cancerous tumors of humans and animals, was caused by bacteria. Studies of how this bacterium, known as *Agrobacterium tumefaciens*, caused tumors in plants led to the discovery, almost a century later, that whenever the bacterium infects plants

FIGURE 1-20 (A) The fire blight bacterium *Erwinia amylovora*. (B) Fire blight on apple trees. (C) Erwin F. Smith. (D) Crown gall, caused by the bacterium *Agrobacterium tumefaciens*. [Photographs courtesy of (A) Oregon State University, and (B) K. Mohan and (D) R. L. Forster, University of Idaho.]

it transfers part of its DNA to the plant and that the DNA is expressed by the plant as if it were plant DNA (see also pages 624–625). The discovery that the bacterium acts as a natural genetic engineer of plants led to the development of this bacterium so that it could be loaded with, and then transfer to plants, DNA segments coding for desirable characteristics, which formed the basis of biotechnology, especially of plants. As with fungal plant pathogens, however, acceptance of bacteria as causes of disease in plants was slow. For example, as late as 1899, Alfred Fischer, a prominent German botanist, rejected the results of Smith and others who claimed to have seen bacteria in plant cells.

Viruses

At about the same time that more diseases of plants were shown to be caused by bacteria, the Dutchman Adolph Mayer (Fig. 1-21A), in 1886, injected juice obtained from tobacco plant leaves showing various patterns of greenish yellow mosaic (Fig. 1-21B) into healthy tobacco plants and the latter then developed similar mosaic patterns. Because no fungus was present on the plant or in filtered juice, Mayer concluded that the disease was probably caused by bacteria. In 1892, however, Ivanowski showed that whatever caused the tobacco mosaic disease could pass through a filter that retains bacteria, so he concluded that the disease was caused by a toxin secreted by bacteria or, perhaps, by unusually small bacteria that passed through the pores of the filter. In 1898, Beijerinck, by repeating some of these experiments, finally concluded that the tobacco mosaic disease was caused not by a microorganism, but by a "contagious living fluid' " that he called a virus.

No one had any idea, however, what a virus was and what it looked like for another 40 years. The true nature, size, and shape of the virus (Fig. 1-21C) remained unknown for several more decades. In 1935, Stanley added ammonium sulfate to tobacco juice extracted from infected tobacco leaves and obtained as a sediment in the flask a crystalline protein that, when rubbed on tobacco, caused the tobacco mosaic disease. This led him to conclude that the virus was an autocatalytic protein that could multiply within living cells. Although his results and conclusions were later proved incorrect, for his discovery Stanley received a Nobel Prize in Chemistry. In 1936, Bawden and colleagues demonstrated that the crystalline preparations of the virus actually consisted of not only protein, but also a small amount of ribonucleic acid (RNA). The first virus (tobacco mosaic virus) particles were seen with the electron microscope in 1939 by Kausche and colleagues. Finally, in 1956, Gierrer and Schramm showed that the protein could be removed from the virus and that the ribonucleic acid carried all the genetic information that enabled it to cause infection and to reproduce the complete virus. It was shown subsequently that although the nucleic acid of most viruses infecting plants is single-stranded RNA, some viruses have double-stranded RNA, some double-stranded DNA, and some single-stranded DNA.

The search for the cause of the many thousands of plant diseases led to the discovery of at least three more kinds of pathogens and it is likely that others remain to be discovered.

Protozoa

Flagellate trypanosomatid protozoa were observed in the latex-bearing cells of laticiferous plants of the family

FIGURE 1-21 (A) Adolph Mayer. (B) Tobacco leaf showing symptoms of tobacco mosaic. (C) Particles of *tobacco mosaic virus*.

Euphorbiaceae by Lafont in 1909. Such protozoa, however, were thought to parasitize the plant latex without causing disease on the host plant. In 1931, Stahel found flagellates infecting the phloem of coffee trees, causing abnormal phloem formation and wilting of the trees. In 1963, Vermeulen presented convincing evidence of the pathogenicity of flagellates to coffee trees, and in 1976 flagellates were reported to be associated with several diseases of coconut and oil palm trees in South America and in Africa. In recent years, of course, the Myxomycota and the Plasmodiophoromycota, which were previously thought to be fungi, have been transferred to the kingdom protozoa.

Mollicutes (Phytoplasmas)

For nearly 70 years after viruses were discovered, many plant diseases were described that showed symptoms of general yellowing or reddening of the plant or of shoots proliferating and forming structures that resembled witches' brooms. These diseases were thought to be caused by viruses, but no viruses could be found in such plants. In 1967, Doi and colleagues in Japan observed mollicutes, i.e., wall-less mycoplasma-like bodies in the phloem of plants exhibiting yellows and witches' broom symptoms. That same year the same group showed that the mycoplasma-like bodies and symptoms disappeared temporarily when the plants were treated with tetracycline antibiotics. Since then, mycoplasma-like organisms (MLOs) that infect plants have been reclassified as phytoplasmas, and some of them that have helical bodies and can be found in other environments besides plants are known as spiroplasmas.

Viroids

In 1971, studies of the potato spindle tuber disease showed that it was caused by a small, naked, single-stranded, circular molecule of infectious RNA, which was called a viroid (see later). Viroids have been found to be the cause of several dozen plant diseases. Viroids seem to be the smallest infectious nucleic acid molecules. Although more than 40 viroids have been found to infect plants, no viroids have been found that infect animals or humans.

Apparently, however, an even smaller type of infectious agent, called a prion, exists (see later). Prions apparently consist only of a small (~55,000 Da) protein, which is encoded by a chromosomal gene of the host. Prions have been shown to cause the scrapie disease of sheep, "mad cow" disease, and at least three slow-developing degenerative diseases of humans. So far, no prions have been found to infect plants, but there is no obvious reason why they should not.

Serious Plant Diseases of Unknown Etiology

Although pathogens as large and complex as fungi and nematodes or as tiny and simple as viroids and prions have been discovered, there are many severe diseases of plants, particularly of trees, for which we still do not know their real cause, despite years of searching and research. Some of them, such as peach short life in the southeastern United States, waldsterben, or forest decline in central Europe and various forest tree declines in the northeastern and northwestern United States, may be caused by more than one pathogen or by combinations of pathogens and adverse environment. Others, such as citrus blight in Florida and South America, spear rot in oil palm in Suriname and Brazil, and mango malformation in India and other mango-growing countries, seem to have a biotic agent as the primary cause, but the activity of the agent seems to be strongly affected by environmental factors such as soil or temperature. Despite more than 100 years of research on some plant diseases, the causes of these diseases remain unknown.

BOX 5 Koch's postulates

Robert Koch (1843–1910) (Fig. 1-16C) was a medical doctor and a bacteriologist. He was the first to show, in 1876, that anthrax, a disease of sheep and other animals, including humans, was caused by a bacterium that he called *Bacillus anthracis*. He subsequently discovered, in 1882, that tuberculosis and, in 1883, that cholera are each caused by a different bacterium, which led to the general conclusion that each disease is caused by a specific microbe. These experiments confirmed for the first time the germ theory of disease proposed earlier by Louis Pasteur.

Before Koch's experiments, and while Koch himself was carrying out the work on the diseases mentioned earlier, there was confusion and uncertainty about the occurrence and the cause of each disease. Much of the time when bacteria or fungi were isolated from diseased or dead human, animal, or plant tissues, the isolated bacteria or fungi were subsequently shown to be saprophytes, i.e., they coexisted with the microorganism that caused the disease but could not by themselves cause the disease for which they were being considered. Based on his experiences, in 1887, Koch set out the four steps or criteria that must be satisfied before a microorganism isolated from a diseased human, animal, or plant

can be considered as the cause of the disease. These four steps, rules, or criteria are known as "Koch's postulates."

1. The suspected causal agent (bacterium or other microorganism) must be present in every diseased organism (e.g., a plant) examined.
2. The suspected causal agent (bacterium, etc.) must be isolated from the diseased host organism (plant) and grown in pure culture.
3. When a pure culture of the suspected causal agent is inoculated into a healthy susceptible host (plant), the host must reproduce the specific disease.
4. The same causal agent must be recovered again from the experimentally inoculated and infected host, i.e., the recovered agent must have the same characteristics as the organism in step 2.

Koch's rules are possible to implement, although not always easy to carry out, with such pathogens as fungi, bacteria, parasitic higher plants, nematodes, most viruses and viroids, and the spiroplasmas. These organisms can be isolated and cultured, or can be purified, and they can then be introduced into the plant to see if they cause the disease. With the other pathogens, however, such as some viruses, phytoplasmas, fastidious phloem-inhabiting bacteria, protozoa, and even some plant pathogenic fungi that are obligate parasites of plants (such as the powdery mildew, downy mildew, and rust fungi), culture or purification of the pathogen is not yet possible and the pathogen often cannot be reintroduced into the plant to reproduce the disease. Thus, with these pathogens, Koch's rules cannot be carried out, and their acceptance as the actual pathogens of the diseases with which they are associated is more or less tentative. In most cases, however, the circumstantial evidence is overwhelming, and it is assumed that further improvement of techniques of isolation, culture, and inoculation of pathogens will someday prove that today's assumptions are justified. However, in the absence of the proof demanded by Koch's rules and as a result of insufficient information, all plant diseases caused by phytoplasmas (e.g., aster yellows) and fastidious vascular bacteria (e.g., Pierce's disease of grape) were for years thought to be caused by viruses.

Despite the difficulties of carrying out Koch's postulates with some causal agents, they have been and continue to be applied, sometimes with certain modifications, in all cases of disease. They have had and continue to have a tremendous effect in deciding and in convincing others that a particular microorganism is the cause of a specific disease. By attempting to carry out Koch's postulates in all newly discovered diseases, a great deal of work with potential saprophytes has been avoided, while, at the same time, doubt and criticism are reduced to a minimum while confidence in and use of the identification increase greatly and quickly.

BOX 6 Viruses, Viroids, and Prions

Although they have been with us forever, we know relatively little about how these pathogen operate. There are many common characteristics among viruses and viroids. The relationship of prions to others is only in their small size but they are contrasted to the other two in that they do not depend on any kind of nucleic acid (RNA or DNA). Viruses cause numerous severe diseases in all types of organisms, have been studied the longest, and we know the most about them. Viroids cause more than 40 diseases in plants, some of them lethal. Prions seem to affect only humans and animals in which they cause degenerative diseases of the brain, such as the recently much publicized "mad cow disease."

Viruses are submicroscopic spherical, rod-shaped, or filamentous entities (organisms) (Figs. 1-22A–1-22C) that consist of only one type of nucleic acid (DNA or RNA). The nucleic acid is surrounded by a coat consisting of one or more kinds of protein molecules. Viruses infect and multiply inside the cells of humans, animals, plants, or other organisms and usually cause disease.

Viroids were discovered by Diener (Fig. 1-22D) and colleagues in 1971 while they were studying the potato spindle tuber disease (Fig. 1-22E). Viroids are the smallest infectious agents that multiply autonomously in plant cells; they consist only of small, circular RNA molecules (Fig. 1-22F) that are too small to code for even one small protein and therefore lack a protein coat. Viroids infect plant cells and are replicated in their nucleus, using the substances and enzymes of plant cells. Viroids infect only plants and in many of them they usually cause disease. Viroids have not yet been detected in any other kind of organism besides plants.

Prions were proposed for the first time in 1972 by Prusiner (Fig. 1-22G) who, for that and subsequent work, received the Nobel Prize in Physiology or Medicine in 1997. Prions are at first normal small protein molecules produced in nerve and other cells of the brain. Prions become pathogenic, i.e., they cannot carry out their normal functions and, instead, have adverse effects on the brain and cause disease. This occurs when prions are forced by conditions in the brain to change shape (Fig. 1-22H). The change in shape signals the onset of infection. Prions are not associated with any nucleic acid. Abnormal prions appear to increase in number and to cause the appearance of amyloid fibrils and plaques, as well as the appearance of small cavities (Fig. 1-22I) in the brain of diseased animals and humans. Prions have not been observed in plants or other organisms.

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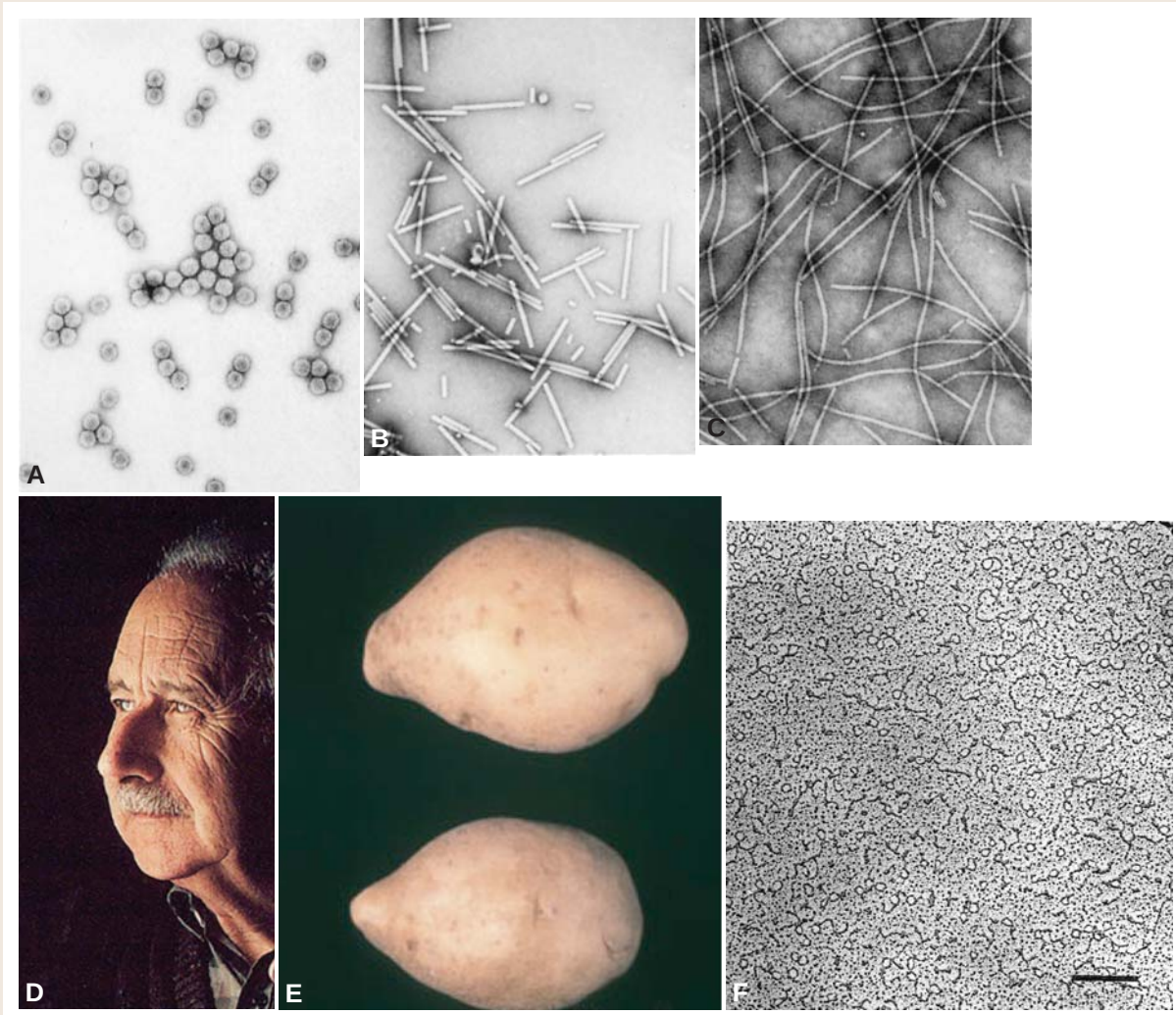

FIGURE 1-22 (A–C) Relative shapes and sizes of plant viruses: spherical, rod shaped, and flexuous. (D) T. O. Diener. (E) Potatoes infected with potato spindle tuber viroid. (F) Circular and linear particles of the coconut cadang-cadang viroid. (G) Stanley Prusiner. (H) Schematic presentation of a normal protein and of a deformed inactive one, i.e., a prion. (I) Plaques in the brain of an animal affected by a prion. [Photographs courtesy of (E) H. D. Thurston, Cornell University, (F) J. W. Randles, University of Adelaide, Australia, and (H and I) S. Prusiner, University of California.]

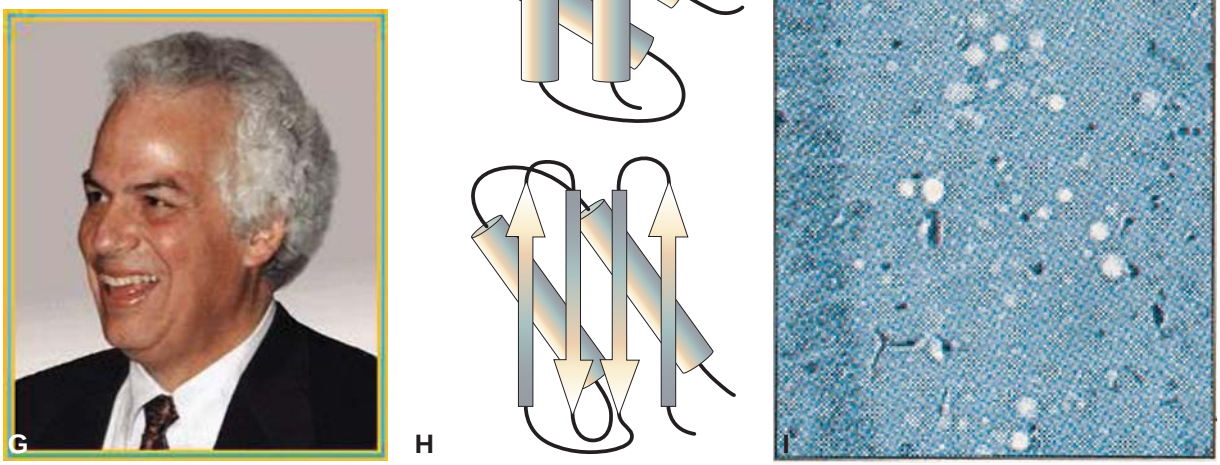

FIGURE 1-22 (*Continued*)

LOSSES CAUSED BY PLANT DISEASES

Plant diseases are of paramount importance to humans because they damage plants and plant products on which humans depend for food, clothing, furniture, the environment, and, in many cases, housing. For millions of people all over the world who still depend on their own plant produce for survival, plant diseases can make the difference between a comfortable life and a life haunted by hunger or even death from starvation. Death from starvation of one and a quarter million Irish people in 1845 and much of the hunger of the underfed millions living in the developing countries today are examples of the consequences of plant diseases. For countries where food is plentiful, plant diseases are significant primarily because they cause economic losses to growers. Plant diseases, however, also result in increased prices of products to consumers; they sometimes cause direct and severe pathological effects on humans and animals that eat diseased plant products; they destroy the beauty of the environment by damaging plants around homes, along streets, in parks, and in forests; and, in trying to control the diseases, people release billions of pounds of toxic pesticides that pollute the water and the environment.

Plant Diseases Reduce the Quantity and Quality of Plant Produce

The kinds and amounts of losses caused by plant diseases vary with the plant or plant product, the pathogen, the locality, the environment, the control measures practiced, and combinations of these factors. The quantity of loss may range from slight to 100%. Plants or plant products may be reduced in quantity by disease in the field, as indeed is the case with most plant diseases, or by disease during storage, as is the case of the rots of stored fruits, vegetables, grains, and fibers. Sometimes, destruction by the disease of some plants or fruits is compensated by greater growth and yield of the remaining plants or fruits as a result of reduced competition. Frequently, severe losses may be incurred by reduction in the quality of plant products. For instance, whereas spots, scabs, blemishes, and blotches on fruit, vegetables, or ornamental plants may have little effect on the quantity produced, the inferior quality of the product may reduce the market value so much that production is unprofitable or a total loss. For example, with apples infected with apple scab, even as little as 5% disease may cut the price in half; with others, e.g., potatoes infected with potato scab, there may be no effect on price in a market with slight scarcity, but there may be a considerable price reduction in years of even minor gluts of produce.

BOX 7 White, dry, and downy vineyards — bordeaux to the rescue!

During the second half of the 1800s, the saying that bad things come in threes found perfect application in the European and particularly the French grape and wine industry. In the 1840s, a condition known to exist on grapes in America but never before observed in Europe appeared first in England and soon after in France: young grape leaves would be covered with spots of white powder (Fig. 1-23A). Later, as the leaf grew in size and age, the white spots would spread and cover most of the leaf. The white mildewy stuff would also get on the berries, which would become dirty gray, wither, and sometimes crack. The condition was called powdery mildew and was later shown to be caused by the fungus *Uncinula necator*. Often, parts of the leaf would turn brown to black and die, while the berries would remain small, discolored (Fig. 1-23B), and unfit for wine production or to be eaten fresh. By 1854, French wine production was reduced by 80% due to the new disease. New grapevines were frantically imported from many countries in the hope that some of them would survive the powdery mildew. Fortunately, at the same time, it was noticed in England that when a mixture of powdered lime and sulfur was dusted on the vines, it significantly protected the leaves and the berries from powdery mildew. This practice became somewhat accepted in France and losses from powdery mildew were reduced significantly.

The early scramble for and importation of foreign vines, however, brought with it a second calamity to the French and European grape and wine industry that was much more disastrous than powdery mildew. In the early 1860s, young leaves on French vines would develop several small galls on the underside (Fig. 1-24A), but then, a few weeks later, all the leaves would turn yellowish to red in early spring and summer and subsequently would wither and fall off (Fig. 1-24B) in July or August. Affected vines produced little or no fruit and the following year they died. The dead, dry leaves gave to the condition the name "phylloxera" (="dryleaf" from the Greek phyllo = leaf, and xera = dry). It was later noted that phylloxera was associated with aphids, some of which fed on the young leaves and induced galls, while many more were found feeding on the roots of grapevines. The aphids not only induced galls on the small roots, they also multiplied quickly and sucked the nutrients out of the roots, killing the roots and, by denying the plant water, caused the leaves to discolor, wither, and fall off. The phylloxera condition was spreading slowly but, in vineyards into which it spread, it had devastating results.

It was determined that phylloxera aphids had probably been brought in from the United States with vines imported for resistance to the powdery mildew problem. The phylloxera aphids, however, did not seem to attack or cause serious damage on American grapevines. So, a new wave of importation of American vines began. These vines were used as rootstocks on which the European varieties were grafted. The degree of resistance of some of the rootstocks to

FIGURE 1-23 Powdery mildew of grape on (A) leaves and (B) grape cluster. White mycelium may cover all green parts, which become dry and brown. (Photographs courtesy of M. A. Ellis, Ohio State University.)

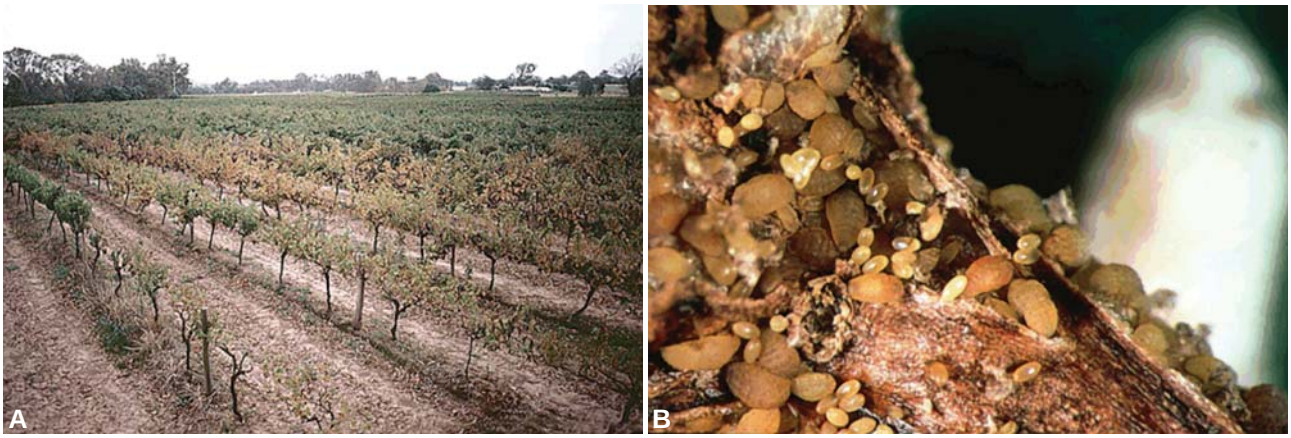

FIGURE 1-24 Phylloxera on grape caused by the grape root aphid. (A) Patch of grapevines showing dry foliage or defoliation due to infection of their roots by the phylloxera aphid. (B) Phylloxera aphids (*Dactylosphaira vitifolia*) feeding on and eventually killing the rootlets of grapevines, thereby causing drying and death of the plants. (Photographs: Queensland Dept. Natural Resources.)

the phylloxera aphids was excellent (Fig. 1-24B) and so the French and other European vineyards could be restored significantly over time.

Unfortunately, however, a third calamity hit the European vineyards while they were just beginning to feel that they had figured out how to escape the destructiveness of phylloxera. In 1878, grape leaves in some French vineyards began to show whitish downy spots on their undersides (Fig. 1-25A), while the upper sides of such leaves corresponding to the underside downy spots became yellow at first and then turned brownish black and died. This condition became known as downy mildew and was shown to be caused by the fungus *Plasmopara viticola*. As the number and size of the spots increased, most or all of the leaf was affected, died, and fell off the vine. Young shoots were also affected, as were young grape clusters, becoming covered with the white downy growth (Fig. 1-25B). Later, they turned brown and eventually shriveled. Berries infected later in the season remained hard compared to healthy ones, exhibited a light green to reddish mottle, and eventually dropped.

The downy mildew spread rapidly within vineyards and from one vineyard to another. It reduced grape yields and quality greatly and killed the young vines in many vineyards. Downy mildew was especially severe and spread the most in cool, rainy weather. Within 5 years of its appearance in France it spread to all the vineyards of that country and into those of adjacent countries. The grape producers in these countries became panicky again. Many scientists showed concern for the problem and interest in finding a solution for it. Some of them used different substances, which they added to the soil or dusted on the vines, trying to protect them from downy mildew. For several years nothing worked. Then one day, the French botany professor Pierre Alexis Millardet (Fig. 1-25C), while walking among the vineyards, noticed that in some of them, the vines of a few rows along the dirt road had a bluish film on their leaves. What was most noteworthy was that these vines seemed to still have all their leaves healthy, whereas vines in rows that did not have the bluish film, the leaves, young twigs, and berry clusters were affected severely by downy mildew (Fig. 1-25D). The owner of the vineyard told him that the bluish film was actually bluestone (copper sulfate), mixed with some hydrated lime to better stick on the leaves. The mixture was sprayed on the vines to create the impression that it was poisonous and in that way to keep passersby from going into his vineyard and taking his grapes. With that information in hand, Millardet went back to his laboratory where he mixed copper sulfate and hydrated lime in various proportions and tried them on downy mildew-affected vines. Finally, in 1885, he found the best combination for the control of downy mildew. This solution (8-8-100) became known as Bordeaux mixture and ushered in the era of control of plant diseases with fungicides. Bordeaux mixture proved to be an excellent fungicide and bactericide and for more than a century was the fungicide used the most throughout the world.

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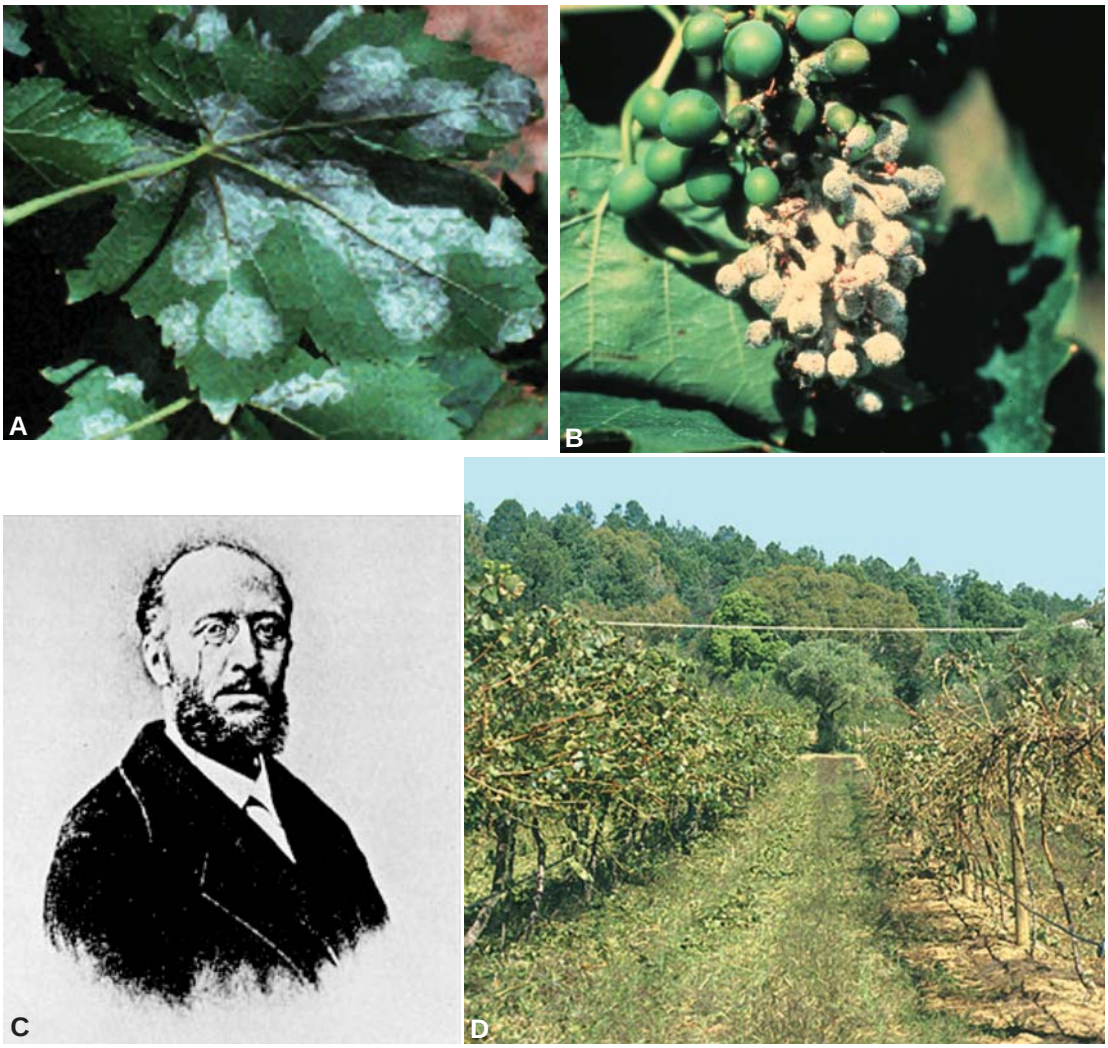

FIGURE 1-25 Downy mildew of grape. Early symptoms on (A) grape leaf and (B) grape cluster. (C) P. Millardet. (D) At left, grapevines exposed to downy mildew but treated with Bordeaux mixture still retain most of their foliage, whereas, at right, unprotected grapes lost almost all of their foliage as a result of downy mildew. [Photographs courtesy of (A) J. Travis and J. Rytter, Pennsylvania State University, (B) University of Georgia, Extension, and (D) G. Ash, Charles Sturt University, Australia.]

Plant Diseases May Limit the Kinds of Plants and Industries in an Area

Plant diseases may limit the kinds of plants that can grow in a large geographic area. For example, the American chestnut was annihilated in North America as a timber tree by the chestnut blight disease, and the American elm is being eliminated as a shade tree by Dutch elm disease.

BOX 8 Familiar trees in the landscape: going, going, gone

In the 19th century, two plant diseases, powdery and downy mildews of grape, and an insect pest of grapes, the phylloxera aphid, each of which alone could have destroyed the European vineyards, spread from North America into Europe. The rediscovery of the use of sulfur against powdery mildew, the discovery of Bordeaux mixture against downy mildew, and the discovery of rootstocks resistant to phylloxera saved the European grape industry in each

case. In the 20th century, Europe returned the favor to North America by giving North America two plant diseases, chestnut blight and Dutch elm disease, each of which killed billions of trees, bringing their respective host species to the brink of extinction. Unfortunately, no good control of these diseases exists even to date, and more of the remaining, at least elm trees, continue to be killed. Another disease, lethal yellowing of coconut palms, has spread through several of the Caribbean islands and adjacent countries, the states of Florida and Texas, west Africa, and elsewhere. Lethal yellowing has destroyed the majority of coconut palms in these areas and, like chestnut blight and the Dutch elm disease, it is still impossible or very difficult to control and continues to kill and threaten the remaining trees with extinction.

Chestnut Blight

There was a time not too long ago that in a broad band of land of the United States, several hundred miles in width and extending from the bottom of the states of Georgia and Mississippi to the top of Maine and Michigan and into Ontario, Canada (Fig. 1-26A), that the most common trees in the forests were the majestic American chestnuts (Fig. 1-26B). They provided timber and chestnuts, the latter serving as a source of food for humans and for wildlife, while the trees served as a habitat for wildlife. Both timber and chestnuts provided a source of income for the local people. The trees had been there apparently forever and looked like they would also last forever.

Then something seemingly minor happened. In 1904, the leaves of a few branches of large chestnut trees and a few young trees in the New York zoo began to turn brown and die. Before anyone could figure out what was happening, many more young trees and branches of older ones died, giving the trees a blighted appearance. From there, chestnut blight spread rapidly through eastern North America so that by the 1920s the blight could be found in the entire natural range of the American chestnut tree. By now, scientists in

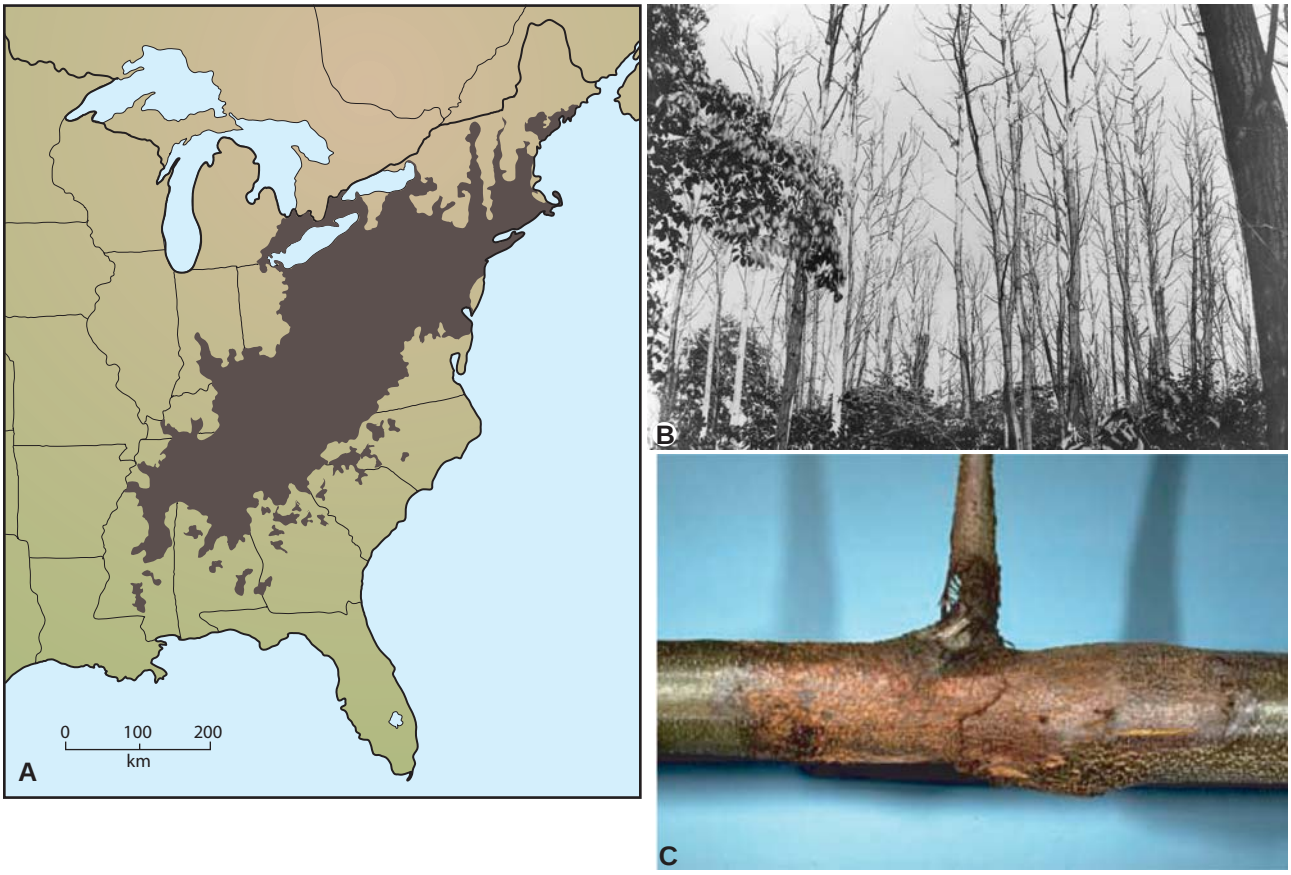

FIGURE 1-26 Chestnut blight. (A) Natural range of American chestnut before the chestnut blight fungus epidemic of 1904–1944. (B) Stand of young, pole-sized chestnut trees devastated by chestnut blight. (C) Chestnut blight canker on trunk of young chestnut tree causing the death of the tree. [Photographs courtesy of (B) W. L. MacDonald, West Virginia University, and (C) R. L. Anderson, U.S. Forest Service.]

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general, and plant pathologists in particular, were quite adept at identifying most causes of plant disease, and chestnut blight was quite easy to diagnose. It was soon shown that chestnut blight is caused by a fungus, *Cryphonectria parasitica*. The fungus attacks and kills the bark of branches and of young trees, causing a canker (Fig. 1-26C) that expands along and around the stem, girdling stems at that point and causing the leaves above the canker to wilt and die. Unfortunately, the fungus produces spores that are carried to other branches and trees by wind-blown rain, by insects, and by birds. By the late 1920s, about three and a half billion American chestnut trees had become infected. Infected trees and branches would produce sprouts from areas below the canker and the sprouts would grow without becoming infected until they were 2 to 4 inches in diameter. At some point, and before they produced any fruit, the fungus would attack and kill them too. That way, although the huge original chestnut trees kept producing new sprouts year after year for many years, their killing by the ever-present fungus finally exhausted the trees and they finally died to their roots. Hardly any trees escaped, making chestnuts the first tree to approach extinction in modern times because of a plant disease caused by a fungus.

Dutch Elm Disease

The American elm grows to be a big, gracefully shaped and beautiful vase-like tree that exists naturally mixed with other hardwoods throughout eastern North American forests and extending into the Great Plains. The elm was soon adopted by early homeowners and town settlers in North America and beautified many a street by being planted in rows on both sides of the street. Then, in 1930, a few elm trees in Cleveland, Ohio, began to show wilting, yellowing, and then browning of the leaves of some branches (Fig. 1-27A). The wilted, brown leaves later fell off and the branch appeared defoliated and dead. More branches showed similar symptoms later that year or the following year, and the entire elm tree usually died (Fig. 1-27B) within 1 or a few years. Trees with similar symptoms were soon observed in some east coast states. The disease became known as Dutch elm disease because, although it had been reported from France in 1917, it was the first report from Holland in 1921 that

FIGURE 1-27 Dutch elm disease. (A) Early symptoms of elm tree showing wilting, curling, and browning of leaves of branch infected with the Dutch elm disease fungus. (B) Advanced symptoms of wilt, defoliation, and death of large branches of tree affected with the disease. (C) Dead elm trees along a road, all killed by Dutch elm disease. [Photographs courtesy of (A) R. J. Stipes, Virginia Tech University, (B) R. L. Anderson, U.S. Forest Service, and (C), E. L. Barnard, Florida Forest Service.]

received all the publicity. The Dutch elm disease spread rapidly in North America, crossing the Mississippi River by 1956 and reaching the Pacific coast states by 1973. In its path, the disease has killed the vast majority of yard, park, and street trees (Fig. 1-27C), although quite a few trees in their natural forest habitat are still free of the disease.

Dutch elm disease is caused by the fungus *Ophiostoma ulmi*. The fungus is carried to healthy elm trees by two elm bark beetles that lay their eggs in weakened or dead elm trees or logs, often those killed by the Dutch elm disease. The eggs hatch and produce larvae that form tunnels, and if the tree or logs are infected with the disease, the fungus grows into and produces spores in the tunnels. The adult beetles then emerge covered with spores of the fungus and look for vigorous young elm branches to feed on. While they are feeding and causing hardly any damage to the elm trees, they deposit spores of the fungus in the feeding wound. The spores germinate and produce mycelium and more spores, both of which spread and multiply in the xylem vessels of the tree and cause the vessels to become clogged. Water and minerals cannot move from the root to the shoots and leaves beyond the point of clogging. The shoots and leaves subsequently wilt and die and, eventually, the entire tree dies.

Lethal Yellowing of Coconut Palms

Lethal yellowing-like symptoms on dying palm trees had been included in brief reports from the Cayman Islands, Cuba, and Jamaica even during the 19th century. In 1955, coconut palm trees in the Key West islands of Florida were noticed to drop their coconuts prematurely. Then, the next inflorescence had blackened tips and set no fruit. Soon, first the lower, older leaves and then the next younger leaves turned yellow and then brown and died. Finally, all the leaves and the vegetative bud died (Fig. 1-28A) and the entire top of the tree fell off, leaving the tall palm trunk looking like a telephone pole (Fig. 1-28B). The lethal yellowing disease was first found in mainland Florida in 1971 and killed 15,000 coconut palm trees by1973, 40,000 by 1974, and, by 1975, 75% of the coconut palm trees in Dade County were dead or dying from the disease. Tremendous losses of palm trees occurred in many other countries. For example, in Jamaica, of six million trees counted in 1961, 90% had been killed by lethal yellowing by 1981. Thousands of hectares of palm trees were killed in Mexico and also in Tanzania, more than a million coconut palm trees were killed in Ghana within 30 years, and more than 60,000, about 50% of the palm trees in Togo, were killed by lethal yellowing by 1964.

The lethal yellowing disease is caused by a phytoplasma, which is a kind of bacterium that lacks a cell wall. The phytoplasma lives and multiplies in the phloem sieve elements of palm trees and causes the lethal yellowing symptoms by clogging some of the sieve tubes and interfering with the transportation of organic foodstuffs out of the leaves and also by producing biologically active substances that are toxic and cause the yellowing and death of the leaves, inflorescence, and vegetative bud of coconut trees. The phytoplasma is spread from diseased to healthy trees by a small plant hopper. The plant hopper sucks up juice

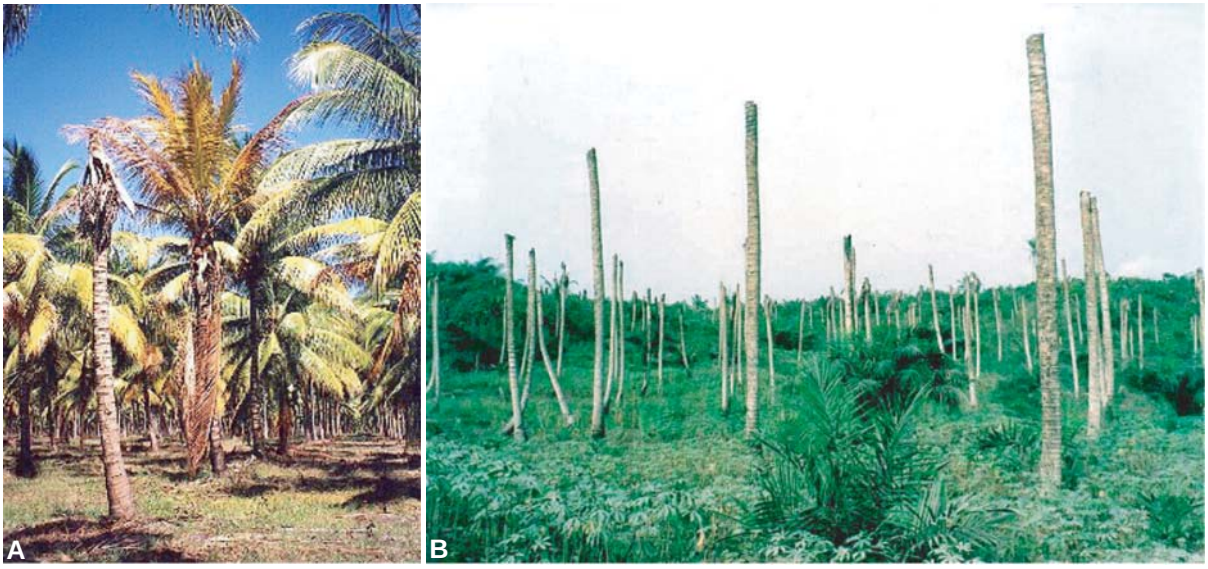

FIGURE 1-28 Lethal yellowing of coconut palm trees. (A) Coconut palms at different stages of the disease, with the disease advancing from the lower fronds upward until the apical bud is killed. (B) Telephone pole-like trunks of coconut palms left after trees were killed by the lethal yellows phytoplasma. (Photographs courtesy of University of Florida.)

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from the phloem of palm trees and, if the tree is infected with the mycoplasma, the plant hopper sucks up some phytoplasmas also. When the plant hopper lands and feeds on a healthy palm tree, it transmits some of the phytoplasmas it carries into the phloem sieve elements. Once in the phloem cells, the phytoplasmas multiply and move throughout much of the phloem of the tree and cause the tree to develop the symptoms of lethal yellowing and to die.

Oak Wilts and Sudden Death

Oaks have been killed for decades by oak wilt (see page 532) caused by the fungus *Ceratocystis fagacearum*, but its spread and development are slower than the Dutch elm disease of elm. At the same time, the oak population is larger and distributed more widely compared to elm. Recently, different species of *Phytophthora* have been attacking and killing oak trees in California, Oregon, Europe, and elsewhere (see pages 418). The progression of these epidemics is hard to predict, but the loss of thousands of oak trees is certain.

Butternut Canker

Butternut trees are native to eastern North American forests and their wood has been used for furniture and for carving. In 1967, butternut trees in Iowa were observed to have multiple cankers on branches and stems and to subsequently die from the disease. Soon afterward, the disease was found to occur widely in the forests of the southeastern coastal region and was shown to be caused by the imperfect fungus *Sirococcus clavigignenti-juglandacearum*. Contrary to chestnut trees killed by chestnut blight, in butternut trees, the canker fungus infects both young and old trees through wounds. Because butternut trees do not sprout after their stem is killed, they are lost entirely. The disease has spread so rapidly that the US Forest Service estimated that about 80% of the butternut trees in the southeast had been killed by the mid-1990s. The remaining survivors were mostly along the banks of streams and rivers, but most of them were also heavily infected and were not reproducing.

Cypress Canker

Cypress trees (*Cupressus sempervirens*) and other species grow in Mediterranean climates, including California, the Mediterranean, and Persia. For more than three millennia they have been valued as ornamentals for their tall, statuesque, columnar shape, as well as for their wood, which is resistant to woodworms, rots, and decays. Cypress trees are extremely long lived, some of them possibly living for more than 2000 years. Many of the world's centers of civilization, such as the Acropolis of Athens, Olympia, Delphi, Florence, and others, and many of the paintings over the centuries derive much of their classic beauty from the real or painted cypress trees in them.

The first cypress canker outbreak was described in California in the mid-1920s, but the disease apparently existed there for more than 10 years before that. The disease then spread inland across the United States and into South America and, apparently, was transported from there across the oceans into the Mediterranean countries, New Zealand, and South Africa so that by now it is believed to be present in most parts of the world where cypress trees grow. Cypress canker or cypress blight is caused by three species of the fungus *Seiridium*, particularly *S. cardinale*. The fungus produces spores (conidia) that infect twigs and small branches through wounds and causes cankers that kill the twigs and branches. Resin flows out of the cracks of cankers while the foliage of infected twigs and branches turns yellowish to red at first, becoming reddish brown as the twigs die. A noticeable dieback of twigs, branches, and tree tops becomes visible at a distance. Heavily infected trees die. Large numbers and large percentages of cypress trees have been killed by the cypress canker fungus in the last few decades. Spread of the disease among the remaining trees continues, possibly at an accelerated rate. As many as one million cypress trees have been killed in central Italy, which includes Florence, with some groves showing more than 45% tree mortality from cypress canker infections. In some of the Greek islands and in parts of the mainland, 70 to 98% of the cypress trees have been killed by this disease.

The Xylella *Outbreak*

The European grape, *Vitis vinifera*, which provides all high-quality table and wine grapes throughout the world, cannot be grown in the southeastern United States because it is devastated by the indigenous xylem-inhabiting bacterium *Xylella fastidiosa*, the cause of Pierce's disease of grape. The disease had been reported in California in the 1880s, but lack of appropriate vectors, appropriate alternate hosts, and timing of unfavorable weather conditions kept the disease under control. As a result, grapes in California and Texas were free of that enemy but, in 1990, the disease was found in Texas where it has spread widely among the vineyards and has caused heavy losses. In 1998, one of its planthopper vectors and the bacterium causing Pierce's disease were found in vineyards of southern California, threatening not only the grape industry, but also many of the ornamental crops of California. *Xylella* bacteria were expected to do well in the California climate, but the absence of an effective vector of the bacteria provided protection and comfort to its agricultural industry. Now that the bacteria and one of their vectors have been brought together in that state, the California grape industry, and possibly its ornamentals, will probably never be the same again.

Plant diseases may also determine the kinds of agricultural industries and the level of employment in an area by affecting the amount and kind of produce available for local canning or processing. However, plant diseases are also responsible for the creation of new industries that develop chemicals, machinery, and methods to control plant diseases; the annual expenditures to this end amount to billions of dollars in the United States alone.

Plant Diseases May Make Plants Poisonous to Humans and Animals

Some diseases, such as ergot of rye and wheat, make plant products unfit for human or animal consumption by contaminating them with poisonous fruiting structures (Fig. 1-29).

BOX 9 Ergot, ergotism, and LSD: a bad combination

For centuries, if not for millennia, people and domestic animals from northern Spain to Russia, and probably elsewhere, suffered periodically from a variety of symptoms ranging from reddening and blistering of the skin to a burning sensation, to excruciating pain in the lower abdomen, muscle spasms, trembling, shaking, and convulsions, hallucinations and permanent insanity, gangrene and loss of fingers and limbs, and, occasionally, death. As a result of the initial burning sensation afflicted persons felt, the disease became known as "devil's curse," "fire," or "holy fire." In 1093, following a series of years of severe outbreaks of the disease, a religious order was formed in southern France to help those suffering from the disease. Because the patron saint of the order was Saint Anthony, the disease became known as "St. Anthony's fire." The disease varied in severity and occurrence from year to year and appeared to affect poor people more often than the well-to-do.

The disease seems to have existed since ancient times. It was described in China as early as 1100 B.C., in Assyria in 600 B.C., and was reported to severely affect the troops of Julius Caesar in one of his campaigns in France. Actually, France has experienced several serious epidemics of "holy fire," including the well-documented ones of 857, of 994 (which is said to have killed between 20,000 and 50,000 people), and of 1093. It is speculated that the Salem witchcraft trials in Salem, Massachusetts, in 1692, may indeed be the result of the "holy fire" disease caused by the consumption of ergot-contaminated flour. In 1722, 20,000 soldiers of the army of Peter the Great of Russia died from consuming bread made from severely infected wheat. Outbreaks of "holy fire" occurred even during the 20th century. For example, in 1926–1927 in Russia, as many as 10,000 people were affected by the disease, more than 200 cases were reported in 1927 in England, and more than 200 people were affected in 1951 in Provence, France, 32 of them becoming insane and 4 dying, all from eating bread made from ergot-contaminated wheat flour.

St. Anthony's fire is known today as ergotism and is the result of people and animals consuming grain coming from cultivated cereals and wild grasses infected with one of several ergot-producing fungi. Ergot (from the French "argot," which means a spur) is the fruiting structure produced by *Claviceps purpurea* and related fungi in place of the seed of the plant (Figs. 1-29A–1-29D) and contaminates the grain after harvest. Ergot is also the name of the disease of cereals and grasses caused by this and related fungi. Ergot, the plant disease, can reduce grain yields significantly, as each ergot replaces completely the kernel that it infects. Most of the damage to the crop, however, is because it makes the rest of the crop unfit for human or animal consumption unless the ergots are removed.

Ergots contain a number of potent alkaloids and other biologically active compounds that affect primarily the brain and the circulatory system. The best known of the ergot alkaloids is lysergic acid diethylamide, the infamous LSD (Fig. 1-29E) that was widely used as a hallucinogen by the hippie culture of the 1960s. Depending on the weather, the host plant (wheat, rye, barley, etc.) and the species of the ergot-forming fungus, the amount of ergot in the field and in the harvested grain may vary, as does the frequency and severity of the symptoms of ergotism (Figs. 1-29F and 1-29G). Rye, which is often consumed by animals and poor people, is the most frequent host of ergot, whereas wheat, preferred by the rich, is the least frequent host of ergot. The property of ergot alkaloids to constrict blood vessels and cause gangrene in humans and animals that consumed food contaminated with ergot sclerotia was put to good use by doctors and midwifes who used ground ergots at the wound to stop excessive bleeding occurring at childbirth and at severe accidents.

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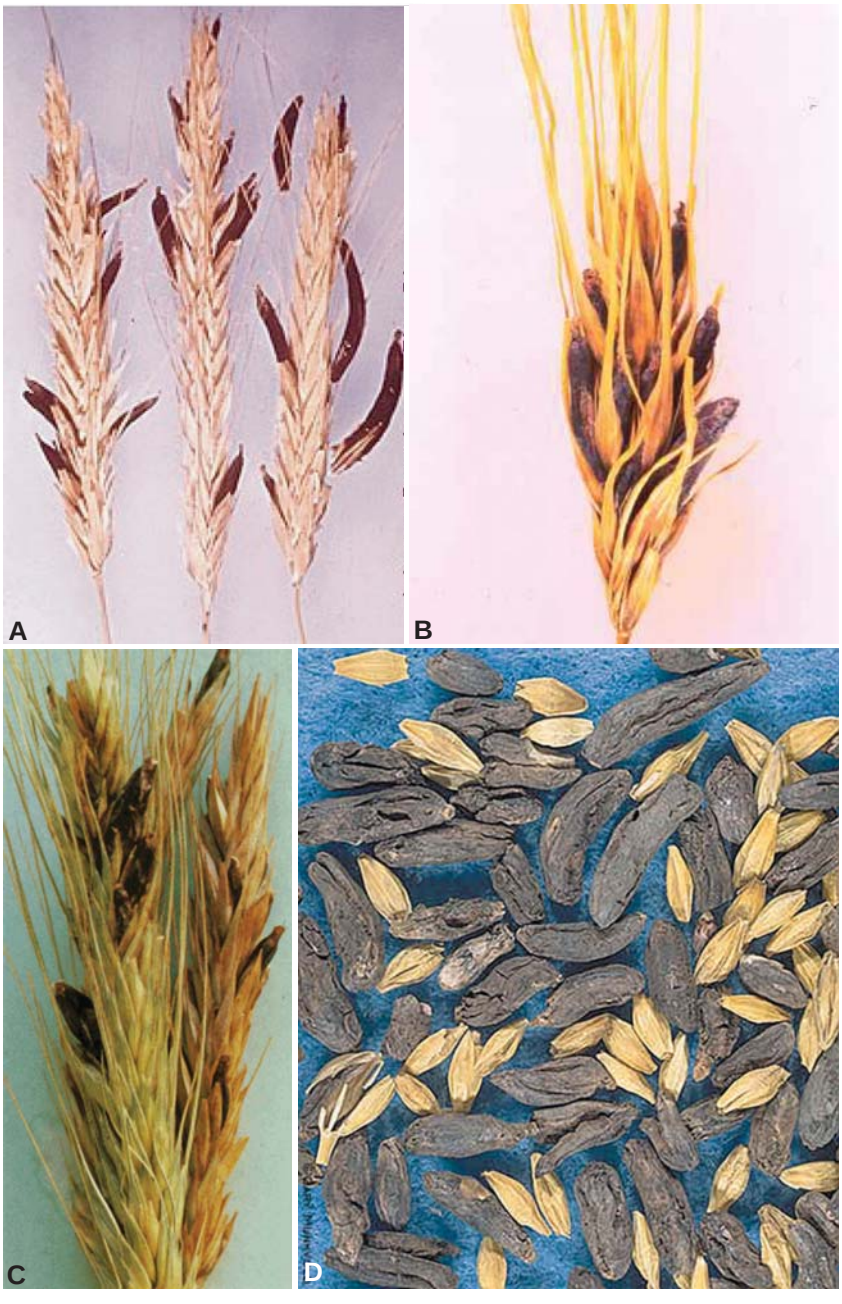

FIGURE 1-29 Ergot of cereals. Ergot sclerotia replacing the kernels in the heads of (A) rye, (B) barley, and (C) wheat. (D) Ergot sclerotia from barley mixed with healthy barley kernels. (E) The chemical formula of LSD found in ergot sclerotia. (F) Calf legs showing hemorrhage caused by consumption of feed containing ergot sclerotia. (G) A sketch of several people, some of whom had become maimed as a result of eating bread containing ground ergot sclerotia. [Photographs courtesy of (A–C) I. R. Evans, WCCPD, (D) G. Munkvold, Iowa State University, (F) Department of Veterinary Science, NDSU, and (G), Breugel, 16th Century, Art History Museum, Vienna.]

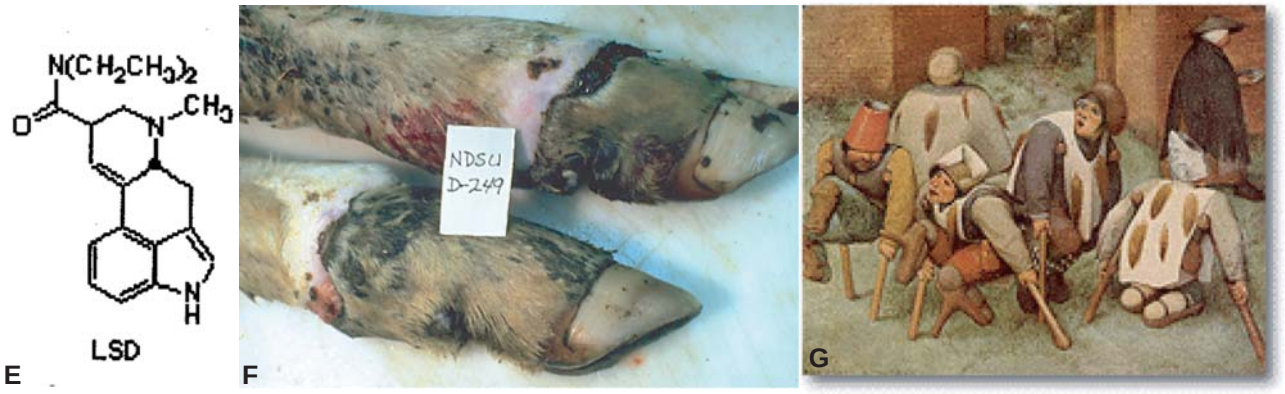

FIGURE 1-29 (*Continued*)

BOX 10 Mycotoxins and mycotoxicoses

Many grains (Figs. 1-30A–1-30D) and sometimes other seeds and also plant products such as bread (Fig. 1-30E), hay, purees, and rotting fruit (Fig. 1-30F) are often infected or contaminated with one or more fungi that produce toxic compounds known as mycotoxins. Animals or humans consuming such products may develop severe diseases of internal organs, the nervous system, and the circulatory system and may die. Also, many pasture grasses are infected with certain endophytic fungi that grow internally in the plant (Fig. 1-30G) and, although they do not seem to seriously damage the grass plants, they produce toxic compounds that cause severe diseases in the wild and domestic animals that eat the plants. Similarly, toxic and sometimes lethal to animals are some grasses whose seeds are infected with bacteria carried there by a nematode; these bacteria are often themselves infected with a virus (bacteriophage) that induces the bacteria to produce compounds very toxic to animals.

Ergotism is an example of a mycotoxicosis caused by food and feed made extremely unhealthy by mycotoxins produced by the fungus *Claviceps purpurea*. Ergotism causes very direct and dramatic symptoms and has been known for many centuries, if not millennia. There have been, however, innumerable other cases in which people or animals became chronically or acutely ill by eating food or feed that contained unsuspected toxic substances. The existence and identity of the toxic substances had remained unknown, the sources of such unsafe food and feed had been little noticed, and the ailments affecting humans and animals remained unexplained. It was not until the 1960s that a severe disease of young turkey birds was shown to be caused by moldy feed and called attention to the importance of mycotoxins in the health of people and animals.

Mycotoxins are toxic fungal metabolites that are released by relatively few but universally present fungi growing on grains, legumes, and nuts. Such produce, especially when harvested while still containing a high percentage of moisture or if it is damaged and stored at relatively high humidity, becomes moldy, i.e., it supports the growth of mycotoxin-producing fungi. Such moldy produce is likely to carry high concentrations of mycotoxins. Several of the mycotoxins are proven carcinogens, may disrupt the immune system, and may retard the growth of animals or humans that consume them. Even very small amounts of mycotoxins bring about the detrimental effect of mycotoxins on the immune system and metabolism of humans and animals, thereby posing a continuous health hazard. At higher concentration, which occur often on moldy produce, mycotoxins cause visible clinical symptoms (mycotoxicoses) in both humans and animals in the form of nervous agitation, dermal and subcutaneous lesions, impaired growth, damage to kidneys and liver, cancer, and others symptoms. Mycotoxins and mycotoxicoses are described in greater detail on page 559–560.

Although the last recorded outbreak of gangrenous ergotism occurred in Ethiopia in 1978, it was not until 1960 that the first general interest in mycotoxicoses was shown when the so-called "turkey X disease" appeared in farm animals in England. It was eventually shown that the disease was caused by feed contaminated with aflatoxins, and when these were shown to cause cancer in the liver of humans and animals, interest in mycotoxins skyrocketed. Aflatoxins are extremely toxic, appear in the milk of animals consuming contaminated feed, attack primarily the liver, and are mutagenic, teratogenic, and carcinogenic. In the last several decades, several outbreaks of aflatoxicosis have occurred in tropical countries where many adults in rural populations often consume moldy corn. Blood examinations in adults and children living in some tropi-

continued

FIGURE 1-30 Mycotoxin-containing plant products infected with mycotoxin-producing fungi. (A) Portion of ear of corn infected with *Aspergillus*. (B) Damaged corn kernels infected heavily with mycotoxin-producing *Gibberella fungi*. Wheat (C) and rye (D) kernels from fields infected heavily with the wheat scab-causing *Fusarium* spp. (E) Bread infected with *Aspergillus*, *Penicillium*, and other fungi. (F) Orange fruit infected with *Penicillium*. (G) Fluorescent mycelium of an endophytic fungus in a grass plant in which it produces mycotoxins. [Photographs courtesy of (A) P. Lipps, Ohio State University, (B) R. W. Stack, North Dakota State University, (C and D) WCCPD, and (G) A. DeLucca, USDA.]

cal areas and showing various symptoms of varying intensity have revealed the presence of aflatoxins in them, with significant seasonal variations.

In addition to aflatoxins produced by the two aforementioned species of *Aspergillus*, several other equally toxic mycotoxins, e.g., ochratoxins, are produced by these and by other species of *Aspergillus*, by *Penicillium*, and by other fungi. Ochratoxins occur in cereals, coffee, bread, and in many preserved foods of animal origin. About 20,000 people in the northern Balkans seem to be suffering from diseases caused by chronic exposure to ochratoxin. Poisoning from moldy sugar cane is caused by a mycotoxin produced by species of *Arthrinium*, and in one rural area in China it affected more than 800 persons who had ingested moldy sugar cane. *Aspergillus* and *Penicillium* are extremely common in nature and are almost always present to some extent in any feed and in most foods. Aflatoxins are the most common mycotoxins, but even more potent mycotoxins, e.g., patulin, roquefortin C, and others, are also produced by species and strains of *Penicillium*.

A number of potent mycotoxins, the trichothecins, are produced by several species of *Fusarium* and, to a lesser extent, by species of *Trichoderma*, *Trichothecium*, *Myrothecium*, and *Stachybotrys*. The most common trichothecin is deoxynivalenol, also known as vomitoxin. Another type of mycotoxin, zearalenone, is produced by somewhat different species of *Fusarium* (*F. graminearum*). Vomitoxin and zearalenone often occur together, especially in scabby wheat and in corn infected with *Gibberella* ear rot, but they have also been found in moldy rice, cottonseed, flour, barley, malt, beer, and other foods. In addition to humans, vomitoxin and zearalenone affect cattle, swine, chickens and other birds, cats, dogs, and fish. Individuals fed contaminated food or feed over a period respond by vomiting, refusal to eat, suppression of their immune system, diarrhea, loss of weight, and low milk production in the case of cows. A still different group of mycotoxins, called fumonisins, are produced by *Fusarium verticillioides* (*F. moniliforme, F. proliferatum*) and related species, primarily in corn and corn-based products. Fumonisins affect all or most of the animals affected by the other *Fusarium* toxins but they also affect and are particularly toxic to horses. In horses, low concentrations of fumonisins cause liquefaction of the brain, resulting in the "blind staggers" and "crazy horse disease" in which horses display blindness, head butting and pressing, constant circling and being agitated, and finally die. In swine, fumonisin attacks the heart and the respiratory system, in which it causes swellings, and it also causes lesions in the liver and pancreas. In humans, fumonisins have been linked to cancer. In the last 10 years, outbreaks of fumonisins in feed or food have been reported in several states from Arizona to Virginia and from South Carolina to the upper Midwest and in some Canadian provinces.

In most of the cases just mentioned, most of the damage is caused by the mycotoxins in food or feed consumed by humans and animals. However, for people and animals spending considerable time surrounded by moldy food or feed, there is the added danger of directly breathing spores of these fungi. It is not clear how detrimental to their health this is, but humans and animals, especially horses, exposed to spores of *Stachybotrys chartarum* develop irritation of the mouth, throat and nose, shock, skin necrosis, decrease in leukocytes, hemorrhage, nervous disorder, and death. *Stachybotrys* grows on straw and feed and on moist surfaces on walls and in air-conditioning ducts and is considered one of the most important causes of the "sick building syndrome."

Plant Diseases May Cause Financial Losses

In addition to direct losses in yield and quality, financial losses from plant diseases can arise in many ways. Farmers may have to plant varieties or species of plants that are resistant to disease but are less productive, more costly, or commercially less profitable than other varieties. They may have to spray or otherwise control a disease, thus incurring expenses for chemicals, machinery, storage space, and labor. Shippers may have to provide refrigerated warehouses and transportation vehicles, thereby increasing expenses. Plant diseases may limit the time during which products can be kept fresh and healthy, thus forcing growers to sell during a short period of time when products are abundant and prices are low. Healthy and diseased plant products may need to be separated from one another to avoid spreading of the disease, thus increasing handling costs.

The cost of controlling plant diseases, as well as lost productivity, is a loss attributable to diseases. Some plant diseases can be controlled almost entirely by one or another method, thus resulting in financial losses only to the amount of the cost of the control. Sometimes, however, this cost may be almost as high as, or even higher than, the return expected from the crop, as in the case of certain diseases of small grains. For other diseases, no effective control measures are yet known, and only a combination of cultural practices and the use of somewhat resistant varieties makes it possible to raise a crop. For most plant diseases, however, as long as we still have chemical pesticides, practical controls are available, although some losses may be incurred, despite the control measures taken. In these cases, the benefits from the control applied are generally much greater than the combined direct losses from the disease and the indirect losses due to expenses for control.

Despite the variety of types and sizes of financial losses that may be caused by plant diseases, well-informed farmers who use the best combinations of

available resistant varieties and proper cultural, biological, and chemical control practices not only manage to produce a good crop in years of severe disease outbreaks, but may also obtain much greater economic benefits from increased prices after other farmers suffer severe crop losses.

BOX 11 The insect-pathogen connection: multifafaceted and important

Insects and similar organisms, such as mites and nematodes, are involved intimately and commonly in the facilitation, initiation, and development of many biotic and abiotic plant diseases. Some insects, e.g., gall-forming aphids and some mites, cause disease-like conditions in plants on which they feed. The importance of insect involvement in the development of pathogen-induced plant disease is so great that it can hardly be exaggerated. For some reason, however, it does not receive sufficient coverage in textbooks and in courses of plant pathology. Insects become involved in disease development in plants primarily through the following four types of action. (1) Insects visit infected plant organs oozing bacteria or fungal spores or plants covered with fungal spores, become smeared with bacteria or spores, and, quite passively, transfer them to other plants where they might cause disease. (2) They cause wounds on plant organs (leaves, fruit, shoots, branches, stems, roots) on which they feed or deposit their eggs and these allow pathogens, primarily fungi and bacteria, to enter the plant. (3) By feeding on plants, especially perennial ones, insects weaken them and make them more vulnerable to attack by some pathogenic fungi. (4) Insects act as vectors of certain pathogens, including a few fungi and bacteria, many viruses, and all phytoplasmas and protozoa. Insects carry these pathogens from diseased to healthy plants where they initiate new disease. These pathogens depend totally on insects for transmission, i.e., in the absence of the insect vectors there is no spread of the pathogen and no new diseased plants.

The first type of incidental transfer of bacteria or fungal spores to other plants or organs where they might cause disease probably involves many types of crawling, walking, or flying insects, such as flies (Figs. 1-31A and 1-31B). Some insects walk through or feed on flower nectar, as, for example, do bees (Fig. 1-31C)) in pear blossoms infected with the fireblight (Fig. 1-31D) bacterium, or on sugars released in infected areas, such as cankers, on stems, or spots or powdery and downy mildews on leaves, or on spots on fruit still on the tree or after harvest. Such insects may include different types of fruit flies, aphids, leafhoppers, beetles, ants, and many others.

Numerous insects feed and cause feeding wounds on various plant organs, e.g., fruits and roots, and several insects cause wounds when they deposit their eggs into such organs. Fungal and, sometimes, bacterial pathogens, such as the soft rot bacterium of potatoes and many other fleshy organs, are facilitated greatly in entering these organs through the wounds made by the insects. For example, the plum curculio beetle (Fig. 1-31E) creates wounds on fruit (Fig.1-31F) during ovipositing. The increased number of entry points for the fungus made on the fruit by insects makes it possible for fungi such as those causing brown rot of pome and stone fruits to be much more damaging in orchards where insect control is poor.

When insects feed on roots, leaves, or shoots of plants, especially perennial ones, the plants not only are wounded in numerous places and allow plant pathogenic fungi and bacteria to enter through the wounds and cause disease, they are also weakened greatly, especially in their ability to mobilize their defenses against pathogens and to protect themselves from becoming diseased. This situation is commonly observed on trees whose roots have been damaged by insects or have been defoliated by insects. In such trees, cankers or root rots, caused by fungi that are normally weak pathogens, develop much more rapidly and cause severe damage or may even kill the entire tree, something that would not have happened in the absence of the damage.

The fourth way in which insects influence the development of disease in plants is by forming close associations with certain pathogens. In such specific insect/pathogen associations, transmission and spread of certain pathogens from diseased to healthy plants depend almost entirely on the availability and involvement of one or a few specific insect vectors. For example, the corn flea beetle (Fig. 1-32A) is the main vector of the bacteria causing bacterial wilt of corn (Fig. 1-32B), whereas the striped and spotted cucumber beetles (Fig. 1-32C) are the main vectors of the cucurbit wilt bacteria (Fig. 1-32D). Similarly, without the vectoring ability of two species of elm bark beetles (Fig. 1-32E) , Dutch elm disease (Fig. 1-32F), which is caused by a fungus, would not possibly occur. Certain insects have also formed symbiotic associations with phloem-inhabiting bacteria such as the citrus greening disease bacteria; with specific xylem-inhabiting bacteria, e.g., the planthoppers that transmit the bacterium that causes Pierce's disease of grapevines; with the xylem-inhabiting nematode causing pine wilt; and with phloem-inhabiting plant pathogenic protozoa causing wilt diseases in coffee and palm trees.

The association of certain insects with specific pathogens, however, has reached its greatest frequency with the plant pathogenic phloem-inhabiting phytoplasmas that cause the yellows, proliferation, and decline diseases of numerous plants (e.g., aster yellows, apple proliferation, coconut palm lethal yellowing), and also with many of the phloem-inhabiting plant viruses. Phytoplasmas are transmitted by the closely related leafhoppers, plant hoppers, and psyllid insects.

Plant viruses, however, are transmitted by one or a few species belonging to the following groups of insects: aphids (Fig. 1-33A) transmit a large number of viruses, such as potato virus Y (Fig. 1-

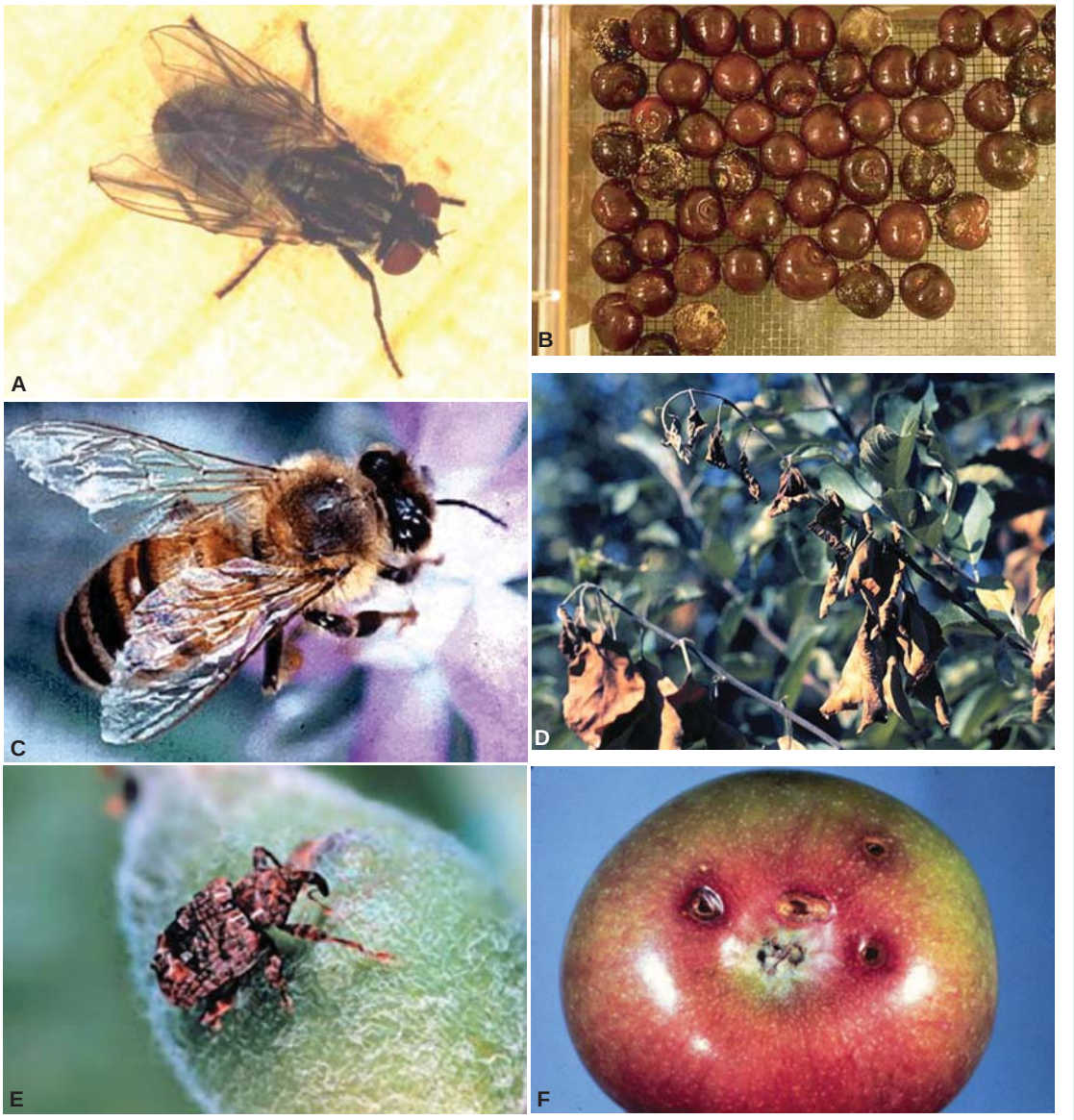

FIGURE 1-31 Examples of insects helping spread plant diseases. Common flies (A) help spread fruit diseases such as brown rot of cherries (B). Bees (C) help spread diseases, such as fire blight of apple and pear (D). Curculio weevil (E) makes holes when ovipositing on fruit (F) that allow fruit-rotting fungi to enter the fruit. [Photographs courtesy of (A and C) University of Florida, (B) J. W. Pscheidt, Oregon State University, (D) T. Van Der Zwet, and (E and F) Clemson University.]

33B); leafhoppers and planthoppers (Fig. 1-33C) vector numerous viruses, such as the rice grassy stunt virus (Fig. 1-33D) (as well as phytoplasmas, spiroplasmas, and xylem and phloem-inhabiting bacteria); and whiteflies (Fig. 1-33E) vector geminiviruses, such as tomato yellow leaf curl virus (Fig. 1-33F). Other specific virus vectors include certain thrips, beetles, and mealybugs. The mechanisms of transmission of viruses by their insect vectors vary considerably. Although all phytoplasmas and most viruses transmitted by leafhoppers are taken up by the insect vector, circulated internally in its body, and multiply in some of its organs before they are injected into the phloem of new hosts, in many of the viruses, especially those transmitted by aphids, the virus is carried on or in the stylet of the vector and through it is deposited in phloem or parenchyma cells of the new host plant.

continued

FIGURE 1-32 Examples of insects serving as specific vectors of many important bacterial and fungal diseases. The corn flea beetle (A) is the vector of Stewart's wilt of corn (B). The striped cucumber beetle (C) is one of two vectors of bacterial wilt off cucurbits (D). The elm bark beetle (E) is one of two vectors of Dutch elm disease (F). [Photographs courtesy of (A) G. Munkvold and (B) M. Carlton, both Iowa State University, (C and D) Clemson University, (E) U.S. Forest Service, and (F) Minnesota Department of Natural Resource Archives.]

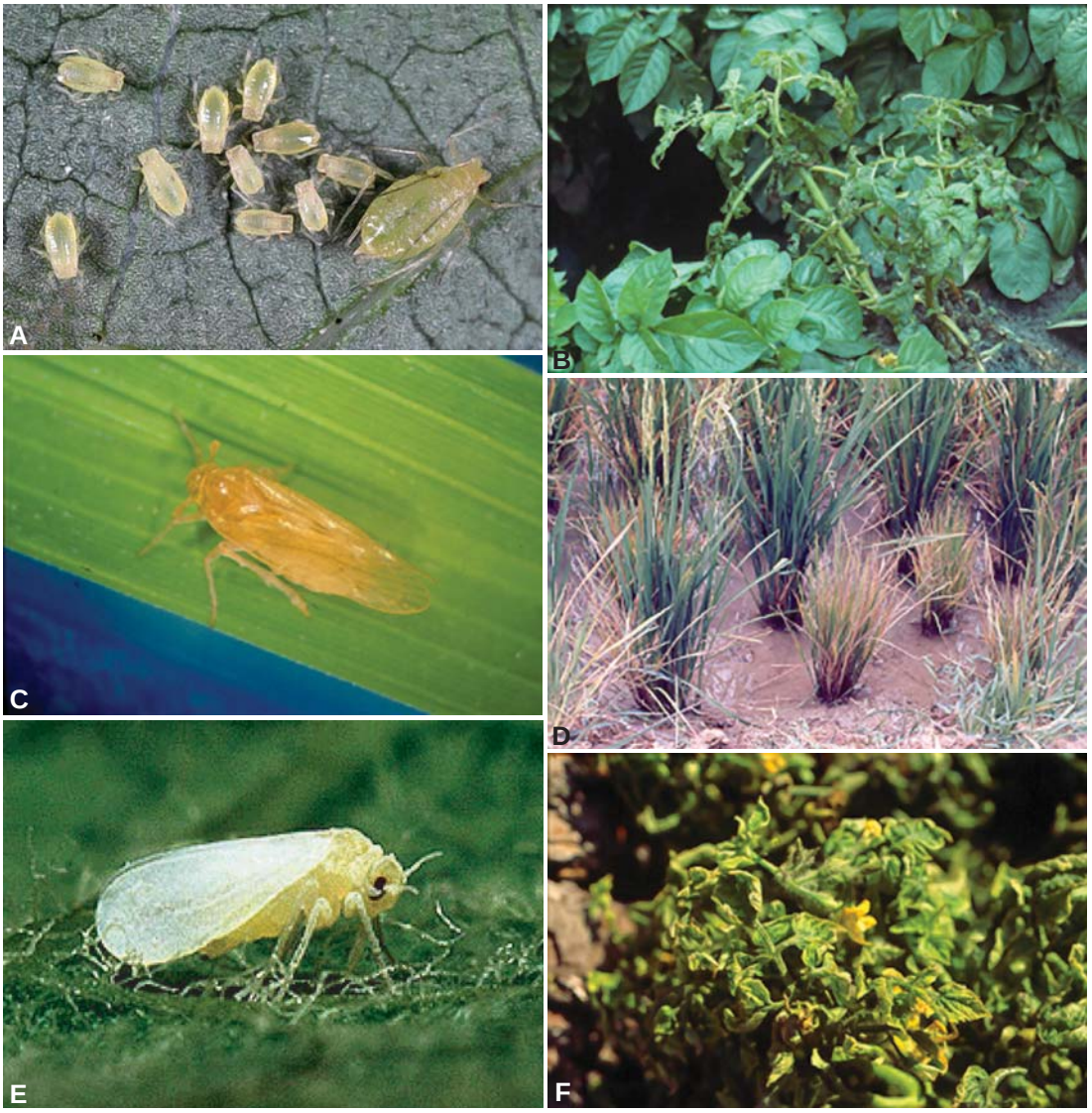

FIGURE 1-33 Examples of insects serving as specific vectors of viruses. Aphids (A) are the most important specific vector of numerous plant viruses such as *potato virus Y* (B). Leafhoppers and related planthoppers (C) are specific vectors for many viruses, such as *grassy stunt virus* (D) and also for phytoplasmas and xylem- and phloem-limited fastidious bacteria. Whiteflies (E) are the specific vectors of many devastating viruses, such as the *tomato yellow leaf curl geminivirus* (F). [Photographs courtesy of (A, B, E, and F) University of Florida and (C and D) H. Hibino.]

PLANT PATHOLOGY IN THE 20TH CENTURY

Early Developments

The Descriptive Phase

As agriculturists, botanists, naturalists, and other scientists, such as physicians, became aware of and familiar with the existence of plant disease and with some of the causes of plant disease, reports began to be published in scientific, popular, and semipopular journals describing numerous plant diseases on a variety of agricultural and ornamental plants. The availability of improved magnifying lenses and of microscopes made possible the detection and description of many fungi, nematodes, and, later, bacteria associated with diseased plants. Development and introduction of techniques for growing microorganisms (fungi and bacteria) in pure culture by Brefeld, Koch, Petri, and others (1875–1912) contributed greatly to plant pathology. In 1887, Koch's

"postulates," which must be satisfied before a particular microorganism isolated from a diseased plant can be accepted as the cause of the disease and not be an unrelated contaminant, had a profound effect on plant pathology. Similarly, improvements in compound microscopes and in plant tissue-staining techniques allowed histopathological and cytological studies of infected plants that revealed the location of the pathogens (mostly fungi, nematodes, and bacteria) in relation to the infected plant cells and tissues. After 1940, the electron microscope made it possible to visualize and describe most viruses and, after 1970, helped detect and describe the mollicutes and viroids.

During the descriptive phase of plant pathology, many observations were also made and reported concerning the biology of the microorganisms involved. Most reports dealt with the types of spores produced by fungal pathogens, the means of spread of pathogens, the location of their survival during winter, the kinds of host plants infected, and so on. Quite often, such observations were correlated with the prevailing environmental conditions, such as rain and temperature, and with differences in disease severity among the various hosts. Different types of control practices, mostly cultural but also some chemical ones, were tried for various diseases. The discovery that sprays with Bordeaux mixture could control the downy mildew of grape encouraged experimentation with this and some other compounds for the control of many diseases on almost all crops.

The Experimental Phase

As the importance of plant diseases and of plant pathology as a new discipline and new profession began to be recognized in the late 1800s, scientists began to be hired as plant pathologists and to be added to the various USDA and state agricultural experiment stations. These scientists began to experiment in all areas of plant pathology. Although new diseases and pathogens continued to be discovered and described, plant pathologists began to ask questions and to design experiments to answer them about how pathogens enter their host plants, multiply, and spread within the plant; the mechanisms of host plant cell death and breakdown; pathogen sporulation; spore dispersal, overwintering, oversummering, and germination; vector involvement; and the effect of environment on disease development, among others. They also began noticing and studying variability among plant species and varieties in disease expression and loss. As knowledge accumulated, experimentation also grew rapidly on ways to control plant diseases and to avoid or reduce the losses from them.

The Etiological Phase

The etiological phase of plant pathology involved observations and experiments aimed at proving the causes (etiology) of specific plant diseases. Although the etiological phase began with the proof of pathogenicity of the late blight fungus on potatoes and of the rust and smut fungi of cereals, etiological studies were facilitated and accelerated greatly by the development of techniques for the pure culture of fungi and bacteria and by the necessity to satisfy Koch's postulates for every disease. Numerous reports in the late 1890s and in the first third of the 20th century dealt with descriptions of the symptoms of thousands of mostly fungal plant diseases on all types of hosts, of efforts to isolate and culture the suspected pathogens, and of subsequent experiments to prove the pathogenicity of the isolated, suspected pathogens. Many of these reports often included information on the losses estimated to be caused by the disease and on experiments about ways that could control the disease.

The etiological phase resumed, continued, and accelerated as new types of pathogens, such as viruses, phytoplasmas, fastidious bacteria, protozoa, and viroids, were discovered. Although the methodologies had to be adapted to the size and properties of each type of pathogen, the goal and the result remained the determination of the etiology of the disease. The etiological phase often depended on, and benefited from, improvements in methodology and instrumentation, such as the electron microscope, special nutrient media, density gradient centrifugation, electrophoresis, the development of serological techniques, the polymerase chain reaction (PCR), and the development of DNA probes and other nucleic acid tests and tools.

The Search for Control of Plant Diseases

As mentioned earlier, in addition to prayers and sacrifices to gods, some minor but realistic recommendations for control of plant diseases were reported in the writings of the ancient Greeks Homer (1000 B.C.), Democritus (470 B.C.), and Theophrastus (300 B.C.). It was not until the mid-1600s, however, that a species or variety was reported to be more resistant to a disease than another related species or variety, although it is assumed that, despite the absence of written reports, growers, knowingly or unknowingly, have been forever using a selection of resistant plants as a control of plant diseases. This is likely to have occurred not only because seeds from resistant and therefore healthier plants looked bigger and better than those from infected susceptible plants, but also because in severe disease out-

breaks, resistant plants were the only ones surviving and, therefore, their seeds were the only ones available for planting.

The earliest use of chemicals for the control of plant diseases probably began in the late 1600s when some farmers in southern England planted wheat seed that had been salvaged from a ship wreck; they noticed that far fewer wheat plants produced from such seed were infected with smut (bunt) than wheat plants produced from other seed. This led some farmers to treat wheat seed with brine (sodium chloride solution) to control bunt. In the mid-1700s, copper sulfate was substituted for sodium chloride, and bunt control improved significantly. This treatment is still used in the poorer parts of the world, although in many countries copper sulfate has been replaced by other, more effective fungicides.

Diseases of fruit and ornamental trees were sometimes too obvious to ignore and although their cause was unknown, several cures, many of them worthless, were proposed. As mentioned earlier, it was noted around A.D. 1200 that a tree can be cured from mistletoe infections if the branch carrying the mistletoe is pruned out. In the mid-1700s, recommendations for the control of cankers included excisions of the canker and the application of grafting wax on the cut area. However, some "scientists" incorrectly recommended the use of vinegar to prevent canker on trees or the use of worthless mixtures of cow dung, lime rubbish from old buildings, wood ashes, and river sand to cure diseases, defects, and injuries of plants. In the early 1800s, lime sulfur and aqueous suspensions of sulfur were recommended for the control of mildew of fruit trees.

The Main Areas of Progress

Chemical Control of Plant Diseases

The introduction from America into Europe of the fungus causing the aggressive downy mildew disease of grape in the late 1870s stimulated a search by several investigators, especially in France, for chemicals that could control the disease. In 1885, Millardet noticed that vines sprayed with a bluish-white mixture of copper sulfate and lime retained their leaves, whereas the leaves of untreated vines were killed by the disease. After trying several combinations, Millardet concluded in that same year that a mixture of copper sulfate and hydrated lime could effectively control the downy mildew of grape. This mixture, which became known as Bordeaux mixture, was soon shown to be equally effective against the late blight of potato, other downy mildews, and many other leaf spots and blights of many different plants. For more than 100 years, Bordeaux mixture was used more than any other fungicide against a wide variety of plant diseases in all parts of the world, and even today it is one of the most widely used fungicides worldwide. The discovery of Bordeaux mixture proved that plant diseases can be controlled chemically and gave great encouragement and stimulus to the study of the nature and control of plant diseases.

In 1913, organic mercury compounds were introduced as seed treatments, and such treatments were routine until the 1960s when all mercury-containing pesticides were banned because of their toxicity. In the meantime, in 1928, Alexander Fleming (Fig. 1-34) discovered the antibiotic penicillin. This was effective against bacteria causing diseases of humans and animals but was not particularly effective against bacterial diseases of plants. Besides, the demand for use against bacterial diseases of humans and animals was so great and the antibiotic was so expensive that its use against bacterial diseases of plants was considered unlikely for at least the next 20 years. Penicillin, however, opened a new area for research in the control of plant diseases. In the meantime, in 1934, the first dithiocarbamate fungicide (thiram) was discovered, which led to the development of a series of effective and widely used fungicides, including ferbam, zineb, and maneb. Many other important protective fungicides followed. In 1965, the first systemic fungicide, carboxin, was discovered, and it was soon followed by the introduction of several other systemic fungicides, such as benomyl.

Antibiotics, primarily streptomycin, were first used to control bacterial plant diseases in 1950. Soon after, the antibiotic cycloheximide was shown to be effective against several plant pathogenic fungi. In 1967, tetra-

FIGURE 1-34 Alexander Fleming.

cycline antibiotics were shown to control plant diseases caused by mollicutes; a few years later, tetracycline was shown to control plant diseases caused by fastidious bacteria that live in the xylem of their host plants.

Appearance of Pathogen Races Resistant to Bactericides and Fungicides

In 1954, it was noticed that a few strains of bacteria causing disease in plants were resistant to certain antibiotics, and, in 1963, strains of fungal plant pathogens were found that were resistant to certain protective fungicides. It was in the 1970s, however, when the use of systemic fungicides became widespread, that new isolates/strains of numerous fungal plant pathogens appeared that were resistant to a fungicide that had previously been effective. The appearance of pathogen races resistant to chemicals prompted the development of new strategies in controlling plant diseases with fungicides and bactericides. Such strategies included the use of mixtures of fungicides, alternating compounds in successive sprays, and spraying with a systemic compound in the early stages of the disease and with a broad-spectrum compound in the later stages of the disease.

Public Concern about Chemical Pesticides

It had long been common knowledge that chemical pesticides are toxic poisons. The word pesticide itself means "pest killer." Pests, of course, include bacteria, fungi, insects, weeds, rodents, and other living things that affect humans, animals, or plants adversely. Depending on the kind of pest against which they are effective, pesticides are known as bactericides, fungicides, nematicides, insecticides, herbicides, and so on.

The public assumed at first that pesticides were toxic only against the kinds of pests at which they were aimed. Scientists and users alike felt certain that animals and humans were not affected by pesticides unless they were fed large amounts of pesticides accidentally or intentionally. For a long time, therefore, pesticides were applied liberally on fields, fruits, vegetables, stagnant waters, and even directly on animals and humans to control insects and diseases affecting them. Hundreds of pesticides were produced annually, and many of the newer pesticides were much more toxic than the earlier ones, i.e., they could kill or seriously injure microbes, pests, higher animals, and humans at a much lower concentration and faster than earlier pesticides. Some of the pesticides broke down into nontoxic or much less toxic compounds soon after they were applied and were exposed to air, sun, and moisture. Others, however, such as DDT and the chlorinated hydrocarbons, consisted of persistent molecules that resisted breakdown and remained toxic for many years or indefinitely.

A few voices of concern about using pesticides were beginning to be heard in the 1950s, but the obvious benefits from controlling insects and diseases in plants, animals, and humans were so overwhelming and the assurances of pesticide safety by scientists and pesticide industries so effective that few such concerns reached the wider public. Rachel Carson's (Fig. 1-35) book "Silent Spring," published in 1962, however, vividly described the dangers of polluting the environment with poisonous chemicals and documented several cases of bird and fish deaths to be the results of pesticides being accumulated and concentrated through the food chain. Carson's book generated a great deal of controversy but also a much greater awareness of the possible adverse effects of pesticides. Many scientists at first were quite skeptical and unconvinced of Carson's arguments. Little by little, however, many of them agreed to do research on the issue of safety of pesticides and began testing insects, earthworms, birds, fish, plants, animals, water streams, lakes, and even soil and underground water reservoirs for pesticides. To the surprise of many scientists, pesticides, particularly the persistent types, were found in many of these bodies, sometimes in fairly high concentrations. By that time (mid-1960s), air pollution by automobiles and factories, water and ground pollution with industrial wastes (chemicals, nuclear reactor byproducts), and so on were also becoming issues of concern to the public. The "Environmental Movement" was solidifying, and concerns about environmental pollution of all types began to gain momentum.

FIGURE 1-35 Rachel Carson.

By the mid-1960s, all pesticides containing mercury were banned by the U.S. government, and soon afterward DDT and chlorinated hydrocarbons were also banned. Laws were passed that prohibited the use of pesticides causing cancer in laboratory animals or mutations in microorganisms. All existing pesticides were subjected to a new, stricter review, and those found to be carcinogenic or mutagenic were banned and removed from the market. The uses of many pesticides that continued to be allowed were further reduced as to the crop, dosage, timing, and number of applications, while the interval between last application and harvest was increased. Since the mid-1980s, approximately 85–90% of the pesticides or pesticide uses previously available for plant disease control have been banned by the U.S. government or discontinued by the manufacturers, and it is likely that several of the remaining ones will be banned or withdrawn in the near future. In the meantime, the requirements for less toxic, more specific pesticides have increased, as have the costs of bringing a pesticide to the market. The costs of potential litigation for injury from pesticides have also increased greatly. Much stricter rules have been imposed on pesticide applicators, pesticide applications, and handlers of products treated with pesticides, with each restriction making it safer, but more expensive, to apply pesticides. The current or anticipated lack of a supply of effective pesticides has increased the effort to develop alternative controls. Different controls may be provided by using antagonistic microorganisms (biological control), improving old cultural practices, and developing new ones. Particularly desirable are new control methods that incorporate disease resistance into crop varieties, either by conventional breeding or through genetic engineering technologies, and using nontoxic compounds that activate the natural defenses of plants.

Alternative Controls for Plant Diseases

Concern over the potential toxicity of pesticides and over the continuing loss of appropriate, effective pesticides available for plant disease control has continued to increase since the 1970s. This has led to the reexamination and improvement of many old practices and to the development of some new cultural practices for use in controlling plant diseases. Proper cultural practices include removal of plant debris and infected plant parts, use of seed free of pathogens, crop rotation with plant species that are immune to the kinds of pathogens that affect the other rotation crops, soil fallow, reduced or no tillage, destruction of weeds, fertilization with appropriate amounts and forms of fertilizer, appropriate irrigation, adjusting the time and rate of sowing and date of harvest, and minimizing the influx of pathogen vectors into crops through border plants. The modification of cultural practices, use of resistant varieties, and monitoring of the appearance and development of plant disease epidemics that allow for a reduced use of pesticides have become the basis of "integrated management" of plant diseases.

It was reported early in the 20th century that some soils, through the microorganisms they harbor or through other means, suppress the development of certain diseases caused by soilborne pathogens. After Fleming reported in 1928 that certain fungi, such as *Penicillium*, inhibit the growth of other fungi and bacteria, plant pathologists began searching for nonpathogenic microorganisms that could be applied to plants before or after infection with a pathogen and that would antagonize the pathogen and keep it from infecting the plant. Numerous nonpathogenic microorganisms, mostly fungi and bacteria, have been found that antagonize various plant pathogenic fungi, bacteria, and nematodes, and some of them have been shown to protect the host plant from infection by the pathogen. In the early 1930s, it was shown that infection of a plant with a mild strain of a virus prevented or delayed infection of the plant by a severe strain of the same virus ("cross protection"). It has been shown more recently that even some plant pathogenic fungi and bacteria can be controlled by pretreatment of the plant with an avirulent or hypovirulent strain of the same species.

Biological control of plant diseases with antagonistic microorganisms is practiced to a rather limited extent. The first such control was obtained in 1963 and involved inoculation of the surface of stumps of freshly cut pines with spores of a nonpathogenic fungus (*Phleviopsis gigantea*) that protected them from infection by the fungus (*Heterobasidion annosum*) that causes root and butt rot of pines. In 1972, control of the crown gall bacterium was obtained by preinoculating seeds or roots of transplants of stone fruit trees with a related but nonpathogenic bacterium, and control of the tobacco mosaic virus in tomato fields was obtained by preinoculating tomato seedlings with a nonpathogenic strain of the virus produced by mutating the virus artificially. Experimentally, biological control can be obtained against many plant pathogenic fungi and bacteria infecting foliage or roots in the field or fruits in storage, and also against some nematodes, but field applications are still mostly ineffective. The control of viral diseases by cross protection is used in the tristeza disease of citrus and in some other virus diseases. A new and promising type of biological control of viral diseases, discovered in the late 1980s, uses the introduction of one or several appropriate viral genes into host plants through genetic engineering and expression of these

genes by the host. These genes then prevent or delay infection of the plant by the virus.

Another recent, very exciting and promising means of plant disease control is through the use of pathogenic microorganisms or chemical compounds that cause tiny necrotic lesions in the treated plant and, by so doing, activate the defenses of the whole plant against subsequent infections by pathogens of the same or different types. This has been called systemic acquired (or induced or activated) resistance. In the early 1990s, nontoxic chemical compounds called plant defense activators were synthesized that, when applied to plants, activate the systemic defenses of plants against pathogens without causing necrotic lesions. The first such compound, named Actigard, was market tested with considerable success in 1996.

Interest in the Mechanisms by Which Pathogens Cause Disease

Once it became apparent that fungi and other microorganisms were the causes rather than the results of plant disease, efforts began to understand the mechanisms by which microorganisms cause disease. In 1886, deBary, working with the Sclerotinia rot disease of carrots (Fig. 1-36) and other vegetables, noted that host cells were killed in advance of the invading hyphae of the fungus and that juice from rotted tissue could break down healthy host tissue, whereas boiled juice from rotted tissue had no effect on healthy tissue. DeBary concluded that the pathogen produces enzymes and toxins that degrade and kill plant cells from which the fungus can then obtain its nutrients. In 1905, cytolytic enzymes were reported by L. R. Jones to be involved in several soft rot diseases of vegetables caused by bacteria. In 1915, it was reported that the pectic enzymes produced by fungi (Fig. 1-37A) play a significant role in their ability to cause disease on plants, but it was not until the 1940s that cellulases were implicated in plant disease development.

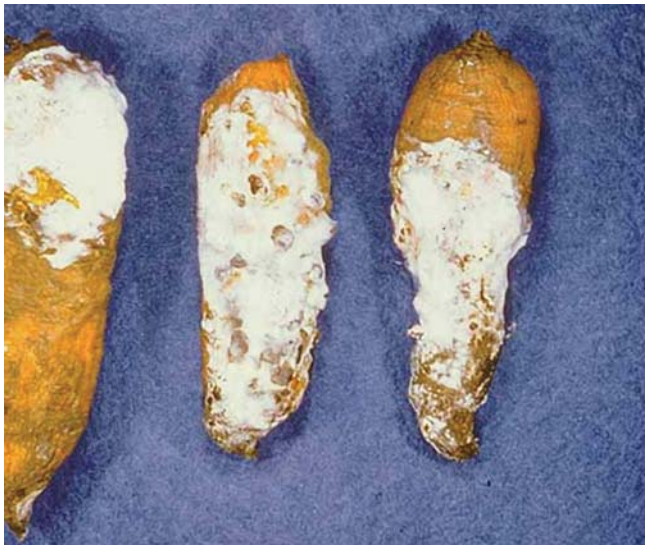

FIGURE 1-36 Sclerotinia white mold of carrots.

After deBary, many attempted to show that most plant diseases, particularly vascular wilts and leaf spots, were caused by toxins secreted by the pathogens, but those claims could not be confirmed. A 1925 suggestion that the bacterium *Pseudomonas tabaci*, the cause of the wildfire disease of tobacco, produces a toxin that is responsible for the bacteria-free chlorotic zone ("halo") (Fig. 1-37B) surrounding the bacteria-containing necrotic leaf spots was confirmed in 1934. The wildfire toxin was the first toxin to be isolated in pure form in the early 1950s. In 1947, a species of the fungus *Helminthosporium* (*Bipolaris*), which attacked and caused blight only on oats of the variety Victoria and its derivatives, was shown to produce a toxin named victorin. This toxin could induce the symptoms of the disease only on the varieties susceptible to the fungus. Many other bacterial and fungal toxins were subsequently detected and identified. The toxins exhibited several distinctive mechanisms of action, each affecting specific sites on mitochondria, chloroplasts, plasma membranes, specific enzymes, or specific cells such as guard cells. In addition, several detailed biochemical studies were carried out to elucidate the mechanisms by which toxins affect or kill plant cells or by which cells of resistant plants avoid or inactivate them.

Early observations that in many diseases the affected plants showed stunting, whereas in others they showed excessive growth, tumors, and other growth abnormalities (Fig. 1-37C), led many investigators to suspect imbalances of levels of growth regulators in diseased plants. In 1926, E. Kurosawa showed that the excessive growth of rice seedlings (Fig. 1-37D) infected with the fungus *Gibberella* could also be produced by treating healthy seedlings with sterile culture filtrates of the fungus. In 1939, the growth regulator produced by the fungus was identified and named gibberellin. By the late 1950s, numerous plant pathogenic fungi and bacteria were shown to produce the plant hormone indoleacetic acid (IAA). In the mid-1960s, a cytokinin was shown to be produced by the bacterium that causes the fasciation (leafy gall) disease of peas and other plants, and the symptoms of the disease could also be reproduced by treating the plants with kinetin, which is an animal-derived cytokinin. In the late 1970s and in the 1980s, detailed studies were made of the mechanisms of disease induction in the *Agrobacterium tumefaciens*-induced crown gall disease of many plants.

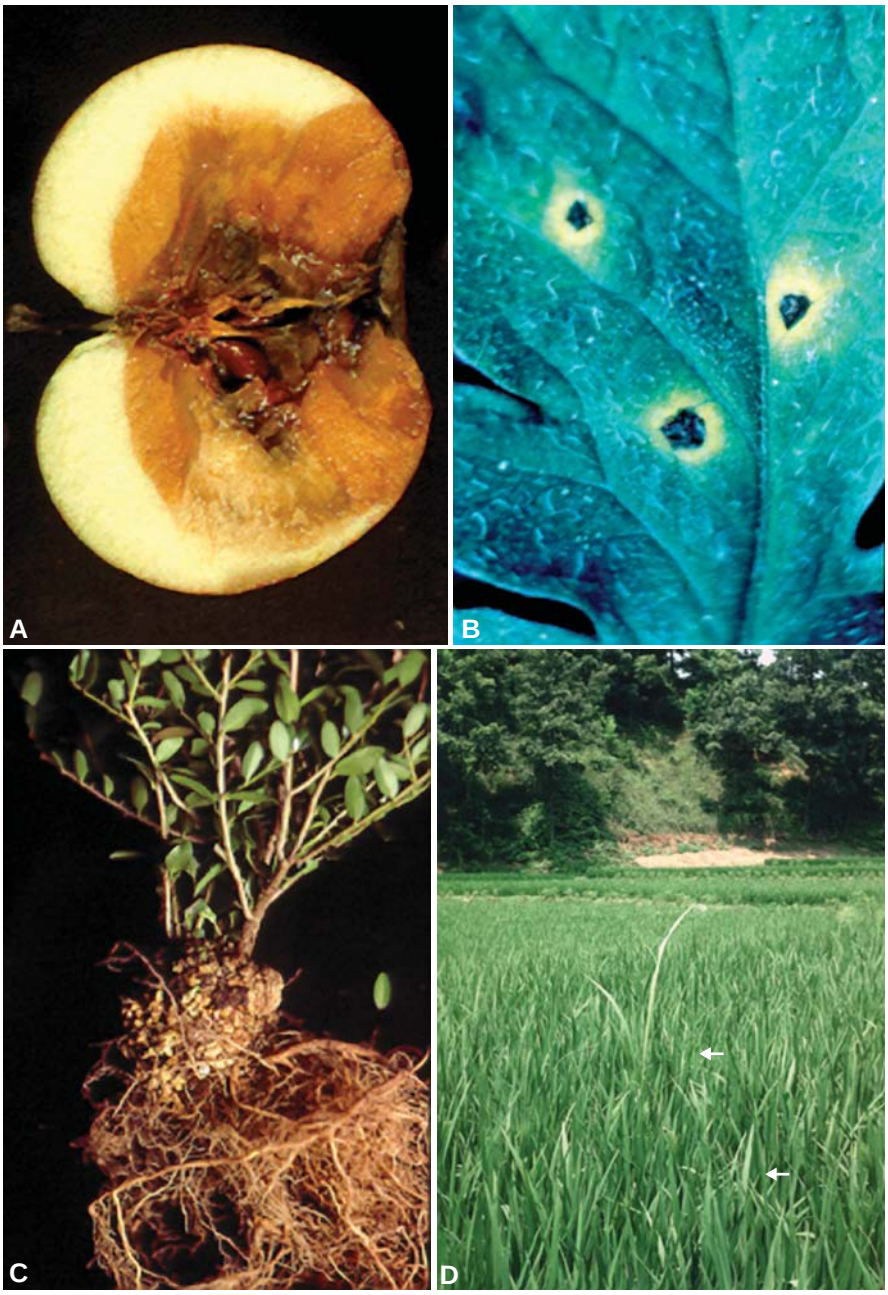

FIGURE 1-37 Chemical weapons used by pathogens in causing disease. (A) Apple infected with gray mold and showing the action of the pectinolytic enzymes ahead of the fungal pathogen. (B) Halo around lesions on tomato leaf show the presence of toxin produced by the bacterial pathogen. (C) Formation of crown gall as a result of excessive amounts of growth regulators produced by the crown gall bacterial pathogen. (D) Excessive growth of rice seedlings is the result of excessive production of gibberellin growth regulators by the fungal pathogen. [Photographs courtesy of (B) R. J. McGovern, (C) University of Florida and (D) R. K. Webster, University of California.]

These studies showed that the bacterium introduces into plant cells a specific part of transforming DNA (T-DNA) of its transformation-inducing plasmid (Ti plasmid). This DNA becomes incorporated into and is transcribed by the plant cell. The T-DNA contains several genes, one of which codes for IAA and one for a cytokinin. When these genes are expressed by the plant cell, the growth regulators they produce lead to uncontrolled enlargement and division of affected plant cells. Depending on the relative concentration of the two

growth regulators, the infection may result in the production of unorganized galls (tumors), partially organized teratomas, or hairy roots.

From the mid-1950s until about 1980, a great many studies were carried out on the effect of infection on the respiration of host cells and on the possible role of altered respiration in plant defenses, and resistance, to infection. Similarly, numerous studies were carried out on the types of host cell enzymes that may be activated on infection, the types and amounts of metabolites (substances) accumulating following infection, and, particularly, the types and amounts of phenolic compounds and phenol-oxidizing enzymes produced following infection. These studies provided a great deal of information on many of the biochemical reactions that go on in plant cells following infection but did not entirely explain the mechanisms by which plants defend themselves against pathogens.

From the early 1970s onward, many studies have been devoted to the elucidation of the numerous metabolic changes associated with the hypersensitive response, i.e., the localized defense reaction of a resistant plant to a pathogen. In the hypersensitive response, numerous enzymes, known as plant pathogenesis-related (PR) proteins, are activated. Some of the PR proteins induce the synthesis of ethylene, which is a plant hormone able to induce many stress responses; some induce the production of oxidative enzymes and proteins involved in cell wall modification and strengthening against pathogen invasion; some synthesize antimicrobial compounds such as phytoalexins; and some are enzymes that attack and dissolve components of the cell wall of the pathogen or are proteinase inhibitors that neutralize specific enzymes of the pathogen. Information on such proteins is, potentially, of great practical significance for possible use to genetically engineer plants, which, upon infection, will produce sufficient amounts of appropriate pathogenesis-related proteins that will result in protecting the plants from becoming diseased.

The Concept of Genetic Inheritance of Resistance and Pathogenicity

In 1894, Eriksson showed that the cereal rust fungus *Puccinia graminis* consists of different biological races that cannot be distinguished morphologically but differ in their pathogenicity to their cereal host; for example, some of them being able to attack wheat, but not the other cereals, such as oats and rye.

In 1902, H. M. Ward recognized the necrotic defense reaction, which E. C. Stakman later (1915), studying it in the cereal rusts, called the "hypersensitive response." In 1964, Z. Klement and colleagues recognized that the hypersensitive response also operates against bacterial plant pathogens. In 1972, a similar necrotic or hypersensitive response was described in animals and was called apoptosis (= falling out); this research showed the existence of many common features in the defense reactions of plants and animals.

In 1905, Biffen reported that the resistance of two wheat varieties and their progeny to a rust fungus was inherited in a Mendelian fashion. In 1909, Orton, working with the Fusarium wilts of cotton, watermelon, and cowpea, distinguished among disease resistance, disease escape, and disease endurance (tolerance). In 1911, Barrus showed that there is genetic variability within a pathogen species; i.e., different pathogen races are restricted to certain varieties of a host species. Soon after, Stakman and colleagues (1914) established that morphologically indistinguishable races of a pathogen within a pathogen species differ in their ability to attack certain varieties. The pathogen races can be distinguished by their ability to infect different varieties within a set of host differential varieties (Fig. 1-38). Their work helped explain why a variety that was resistant in one geographic area was susceptible in another,

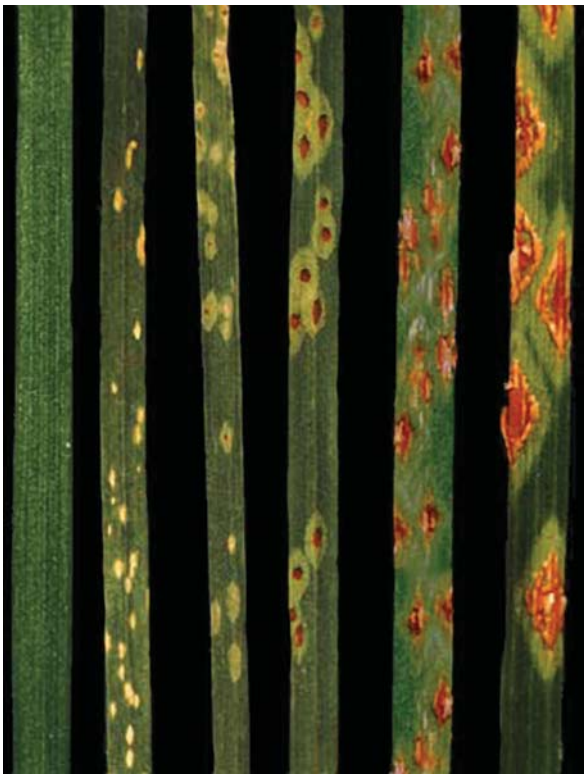

FIGURE 1-38 Differential reaction of leaves of wheat varieties to a race of wheat rust. This test is used to monitor the appearance of new rust races. (Photograph courtesy of USDA.)

why resistance changed from year to year, and why resistant varieties suddenly became susceptible. In all cases the change was due to the presence or appearance of a different physiological race of the pathogen.

The genetics of disease resistance and susceptibility remained obscure until 1946 when Flor (Fig. 1-39A), working with the rust disease of flax, showed that for each gene for resistance in the host there was a corresponding gene for avirulence in the pathogen and for each gene for virulence in the pathogen there was a gene for susceptibility in the host plant (a gene-for-gene relationship).

In 1963, Vanderplank (Fig. 1-39B) suggested that there are two kinds of resistance: one, known as vertical resistance, is controlled by a few "major" resistance genes and is strong but is effective only against one or a few specific races of the pathogen, and the other, known as horizontal resistance, is determined by many "minor" resistance genes and is weaker but is effective against all races of a pathogen species. It has been proposed that each major or minor gene for resistance represents one or several steps in a series of biochemical reactions and that it usually operates in conjunction with several other genes. Together, these genes enable the plant to produce certain types of plant cell substances and structures that interfere with, or inhibit, the growth, multiplication, or survival of the attacking pathogen, and in that way they inhibit, or stop, the development of disease. Some of the plant defense structures and substances exist before the plant comes into contact with the pathogen, but the most effective defense structures and substances are produced in response to attack by the pathogen.

In 1946, E. Gaümann proposed that in many host–pathogen combinations plants remain resistant through hypersensitivity; i.e., the attacked cells are so sensitive to the pathogen that they and some adjacent cells die immediately and in that way they isolate or cause the death of the pathogen. In the early 1960s, it was proposed that, in some cases, disease resistance is brought about by phytoalexins, i.e., antimicrobial plant substances that either are absent or are present at nondetectable levels in healthy plants, but accumulate to high levels in response to attack by a pathogen.

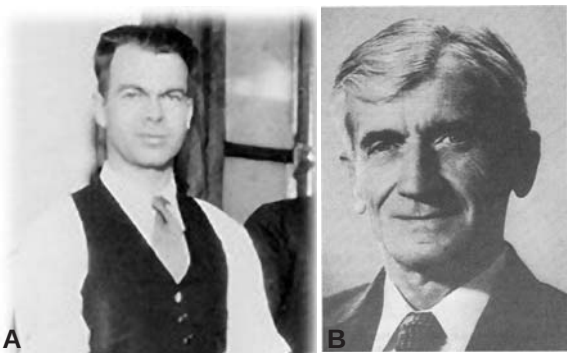

FIGURE 1-39 (A) H. H. Flor. (B) J. E. Vanderplank.

The genetic inheritance of pathogenicity in pathogens has been shown to parallel, and to mirror, that of resistance in plants, as mentioned previously. Some pathogen genes for virulence and even more genes for avirulence have been isolated, and the sequences as well as the products (enzymes, toxins, inhibitors, growth regulators) of several of these genes are also known.

Epidemiology of Plant Disease Comes of Age

Epidemiological observations, i.e., observations concerning the increase of disease within plant populations and how such increases relate to environmental factors, were recorded with many plant diseases as the latter began to be reported. Little effort was made, however, to correlate and utilize such information in controlling plant diseases. From studies of the apple scab disease, Mills in 1944 developed a table listing the duration of rain required at each temperature for apple buds, leaves, and fruit to become infected by the ever-present apple scab fungus. He and others then could use this information to predict whether infection would take place and whether, therefore, control measures (fungicides) should be applied.

It was in 1963, however, that Vanderplank (Fig. 1-39B), through the book "Plant Diseases: Epidemics and Control," established epidemiology as an important and interesting field of plant pathology. In his book, Vanderplank discussed the principles and variables in plant disease epidemics, stated the difference in the development and control of monocyclic and polycyclic pathogens, and described the general structure and patterns of epidemics. A few years later, modeling of plant diseases was introduced, which, through analysis of information on the host, the pathogen, and their interactions, collected at various points in time and under varying environmental conditions, could predict the course of an epidemic. In 1969, the first computer simulation program of plant disease epidemics was published for the fungal-induced early blight disease of tomato and potato. The simulation program was developed by modeling each stage of the life cycle of the pathogen as a function of various environmental conditions designed to stimulate the pathogen. Since the mid-1970s, disease modeling and computer simulation of epidemics have been developed for many diseases and, together with newly developed disease-monitoring instrumentations, have been used in plant disease-

forecasting systems. Disease forecasting has become an important component of integrated pest management (IPM) and has helped reduce the amounts of pesticides applied to crops without reducing yields.

PLANT PATHOLOGY TODAY AND FUTURE DIRECTIONS

Molecular Plant Pathology

Since 1980, great emphasis has been placed on determining the specific molecule and the "genetic connection" of any substance involved in disease development. Because viruses and bacteria are small in size and because a great deal of background information is available on them, more molecular studies have been carried out with them than with the much larger fungi and nematodes. Already the number, location, size, sequence, and function of most or all genes of many viruses are known in detail. Many of these genes have been excised from the virus and have been transferred either to host plants, to which they often convey resistance, or into bacteria, in which they are expressed and the proteins they code for are isolated and studied. Similar transfers have been accomplished with a few bacterial and fungal genes coding for certain pathogenesis-related proteins.

The beginnings of molecular plant pathology can probably be traced to the isolation by W. Stanley in 1935 of the tobacco mosaic virus as a crystalline protein, which he believed to be infectious. Although 2 years later it was shown that the protein also contained a small amount of RNA, it was not until 1956, when Gierrer and Schramm showed that the ribonucleic acid and not the protein of tobacco mosaic virus was responsible for the infection of plant cells and for the reproduction of complete virus particles. In the meantime, in 1941 Beadle and Tatum showed that one gene codes for one enzyme. The following year (1942) Flor showed that a single gene is responsible for pathogenicity in the flax rust fungus and that the rust fungus gene corresponds to a single gene for resistance in the flax plant (the **gene-for-gene concept**). In 1953, Watson and Crick showed that DNA exists in a double helix and their discovery impacted greatly all of biology. In the mid-1960s, studies of tobacco mosaic virus led to the full elucidation of the genetic code according to which specific base triplets of DNA (and RNA) code for a certain amino acid. This was followed by the description in the 1970s through the 1990s of all the genes of tobacco mosaic and of many other viruses.

By the mid-1970s, the studies of *A tumefaciens* revealed that the T-DNA of its Ti plasmid contained several genes of which two, coding for growth regulators, were responsible for the production of tumors (galls) by the infected plants. It was later shown that the two genes could be removed and replaced with one or more genes from other organisms such as plants, other bacteria, viruses, and even animals, genes that could be transferred into and expressed (translated) by the plant cells. This discovery made possible the introduction of foreign genes into plants at will and, combined with tissue culture, which made possible the production of whole plants from single cells, it ushered in the era of genetic engineering of plants. Subsequently, it was discovered that foreign DNA can be introduced into plant cells in several ways, including using viruses as vectors, bombarding plant cells with foreign DNA, and growing plant cells in the presence of foreign DNA. Several viral genes coding for the coat protein or other structural or nonstructural proteins, and some noncoding regions, have been engineered into plants, and many of them have been shown to make the plant more or less resistant to the virus. Also, some bacterial and fungal genes, coding for enzymes that break down the cell wall of the pathogen, have been engineered into plants and have provided the plant with resistance to these pathogens.

In 1984, P. Albersheim and colleagues identified the molecule in the cell wall of the oomycete *Phytophthora megasperma* that acts as the elicitor of the defense response in its soybean host. It was shown later that the elicitor accomplishes this by interacting with a receptor molecule on the plant cells. In the same year, the first avirulence gene was isolated from the bacterium *Pseudomonas syringae* pv. *glycinea* by B. J. Staskawicz and colleagues. These two discoveries helped launch research that improved our understanding of pathogen virulence and plant disease resistance greatly. In 1986, bacterial hypersensitive response protein (hrp) genes were discovered. It was thought at first that the hrp genes were required for bacterial pathogenicity and production of the hypersensitive response; it is known now that they affect the transport of proteins in pathogenic bacteria and also the transport of bacteria into plant cells.

The first practical results of molecular plant pathology in improving disease resistance came in 1986 when R. Beachy and colleagues obtained tobacco plants resistant to tobacco mosaic virus (TMV) by transforming them; i.e., introducing into them the coat protein gene of the virus in a way that the plants could express the gene and produce the virus protein. Such transformed plants are called transgenic, and the resistance they acquire is called pathogen-derived resistance. In 1989, M. B. Dickman and P. E. Kolattukudi transformed a fungus, that normally could enter host plants only through wounds, with a cloned gene coding for the

enzyme cutinase. That enzyme enabled the fungus to penetrate host plants directly through the cuticle, thereby proving that cutinases play a role in the direct penetration of some plants by fungi. Two years later, in 1991, R. Broglie and co-workers showed that plants transformed with the gene that codes for chitinase exhibit enhanced resistance to disease by fungi that contain chitin in their cell walls. In the meantime, in 1990, R. Cheim and colleagues obtained transgenic tobacco plants that expressed increased disease resistance by transforming them with the gene for stilbene synthetase, the enzyme that synthesizes a phytoalexin.

Discoveries in molecular plant pathology came fast and furious in the 1990s. The concept of systemic acquired resistance (SAR) burst onto the scene through the discovery of D. F. Klessig and colleagues and J. Ryals and co-workers that salicylic acid, a relative of aspirin, is associated with SAR. The first fungal avirulence gene (avr9) was isolated from *Cladosporium fulvum* by P. J. G. M. De Wit, while the first plant resistance gene (Hm-1) was isolated from corn by S. P. Briggs and J. D. Walton. The latter also showed that Hm-1 operates by producing a protein that detoxifies the host-selective toxin of the pathogen *Cochliobolus carbonum*. The only resistance gene conferring resistance in tomato to a bacterial pathogen through the hypersensitive response was isolated by G. B. Martin and colleagues in 1993. In subsequent years, dozens of plant disease resistance genes were isolated from many plants. All these genes shared a leucine-rich repeat in the protein they coded for. Tomato plants transformed by B. Baker and co-workers with the tobacco plant resistance gene N, which makes tobacco resistant to tobacco mosaic virus, were also made resistant to the virus, proving that at least some resistance genes may function in species other than the one in which they normally occur. Furthermore, it was shown by V. M. Williamson and colleagues (1998) that a single cloned disease-resistance gene from tomato can confer resistance to both a nematode pathogen and an insect. It was also shown during this period (T. Shiraishi *et al*., 1992) that plant pathogens produce proteins that actively suppress the defense reactions of their host plants. In addition, the avirulence proteins of some pathogens contain signals that allow these proteins not only to be introduced into plant cells, most likely through the bacterial hrp protein system, but also to move into and function in the plant nucleus.

A new type of defense against pathogens was unveiled when it was discovered that many organisms, including plants, fungi, and animals, are capable of "RNA silencing," i.e., of regulating genes based on targeting and degrading sequence-specific RNAs. In plants, RNA silencing has been shown to serve as a defense against virus infections. As would be expected, however, many plant viruses carry genes that encode proteins that suppress the silencing of their RNA by the plant. RNA silencing can be induced experimentally and targeted to a single specific gene or to a family of related genes. It is believed that RNA silencing genes will soon play an important role in engineering resistance into plants.

Advances in molecular plant pathology have also provided a new set of diagnostic tools and techniques that are used to detect and identify pathogens even when they are present in very small numbers or in mixtures with other closely related pathogens. Such tools include detection with monoclonal antibodies, analysis of isozymes or of fatty acid profiles of pathogens, analysis of fragments of their nucleic acids produced by specific enzymes, calculation of percentages of hybridization of their nucleic acids, and determination of nucleotide sequences of the nucleic acids of the pathogens. Since the mid-1980s, segments of DNA (probes), complementary to specific segments of the nucleic acid of the microorganisms, have been labeled with radioactive isotopes or with color-producing compounds and are used extensively for the detection and identification of plant pathogens. Numerous techniques, often referred to by their acronyms, have been developed and are used; some of them are better suited for diagnosing one or more types of pathogens. For at least some pathogens, PCR, with selected differential random sequences of different species, can be effective for the detection and identification of each of these species. At other tests, PCR of sequence segments of rDNA internal transcribed spacer (ITS) regions are used or PCR of other genes or spacers of the fungal DNA is carried out. The product is then differentiated by digestion with restriction enzymes and gel electrophoresis and detection of differential random fragment length polymorphisms (RFLP) or use of PCR together with DNA hybridization in a reverse dot blot hybridization (RDBH) assay using PCR of selected RAPD markers. Reverse transcription PCR (RT-PCR) or immunocapture RT-PCR (IC/RT-PCR), direct binding PCR (DB-PCR), and a combination of PCR and enzyme-linked immunosorbent assay (ELISA) tests are often used successfully, especially for viruses.

An area of molecular plant pathology that is going to pay multiple dividends in the future is that of genomics, i.e., sequencing of the entire genomes of plants and their pathogens. Already, the genomes of the experimental plant *Arabidopsis thalliana*, of several plant viruses and viroids, and of the plant pathogenic bacteria *Ralstonia solanacearum* and *Xylella fastidiosa*, the white rot fungus *Phanerochaete chrysosporium*, and the model nematode *Caenorhabditis elegans* have been sequenced in their entirety. Significant progress has already been made in sequencing the entire genomes of the very destructive plant pathogenic fungi *Magnaporthe grisea*,

cause of rice blast; *Ustilago maydis*, cause of corn smut; *Cochliobolus heterostrophus*, another pathogen of corn; *Botrytis cinerea*, the gray mold of many fruits and vegetables; *Fusarium graminearum*, cause of head scab of wheat; and *Phytophthora infestans*, cause of the blight of potato and of many other pathogens of crops. Once the genomes have been sequenced, it will be easier to locate, identify, compare, isolate, and manipulate the genes for pathogenicity in the pathogens and of resistance in their host plants, as well as manipulate the introduction of them into specific locations of the plant genome where they would be most effective.

The molecular phase of plant pathology is expected to develop a great deal more and to make contributions in ways that we can hardly imagine at present. One area in which molecular plant pathology is expected to contribute greatly and to provide tremendous benefits is the area of detection, identification, isolation, modification, transfer, and expression of genes for disease resistance from one plant to another. Several such resistance genes have already been identified, isolated, transferred into susceptible plants, and, when expressed, made the plants resistant. The possibility that molecular plant pathology can modify and combine resistance genes makes likely the future utilization of resistance genes from unrelated plants or from other organisms, and perhaps even the synthesis of artificial genes for resistance for incorporation into crop plants. The practical implications of such developments cannot be overestimated, as they are likely to revolutionize the control of plant diseases by providing us with cultivars that can resist disease in the presence of the pathogen, without the need to use any pesticides.

ASPECTS OF APPLIED PLANT PATHOLOGY

BOX 12 Plant biotechnology — the promise and the objections

Plant biotechnology can be defined as the use of tissue culture and genetic engineering techniques to produce genetically modified plants that exhibit new or improved desirable characteristics. The desirable characteristics include, among others, better yields, better quality, and greater resistance to adverse factors, including diseases, pests, and environmental conditions such as freezes, drought, and salinity. Plant biotechnology also makes possible the production in plants of useful proteins coded by microbial, animal, or human genes. Plant biotechnology has shown that all of these goals are attainable, at least in the kinds of plants on which they have been attempted. The number of crop, ornamental, and forest plants that have been modified genetically and released by university and industry scientists around the world is in the thousands and continues to grow.

There are numerous cases in which plant biotechnology is used successfully to produce crop plants that avoid or resist certain plant pathogens. Some plants have been rendered resistant to specific pathogens by genetically engineering (transforming) them with isolated specific genes that provide resistance against these pathogens. Transformed plants become resistant by coding for enzymes that mobilize other enzymes that carry out numerous defensive functions, such as breaking down the structural compounds of the pathogen. Several of the enzymes produce compounds in the plant that are toxic to or otherwise inhibit the growth and spread of the pathogen both through the plant and to other plants. Other plants have been transformed with animal (mouse) genes that code for antibodies (plantibodies) against a coat protein of the pathogen. Genetic engineering has been particularly effective in producing plants resistant to viruses by incorporating viral genes in the crop plants that code for virus coat protein, for altered movement protein, or by incorporating in the plant noncoding segments of virus nucleic acid or even segments of the nonsense strand of the virus nucleic acid. Many of these crop plants have been tested for resistance in the field with excellent results.

Practical examples of successful genetic engineering of disease-resistant plants include melon, squash, tomato, tobacco, and papaya crops that are protected from a variety of viral diseases. The success of genetically engineered papaya for resistance to papaya ringspot virus has saved the papaya as a crop in Hawaii and in the Far East (Fig. 1-40). Numerous other cases are still under development. For example, engineering tobacco with a chimeric transgene containing sequences from two different viruses (turnip mosaic and tomato spotted wilt) resulted in new plants resistant to both viruses. Similarly, engineering tomato plants with a truncated version of the gene coding for the DNA replicase of one of the very destructive geminiviruses resulted in plants resistant not only to the virus from which the transgene was obtained, but also to three other viruses. In other work, potato plants engineered with a chimeric gene encoding two insect proteins exhibiting antimicrobial activities showed significant resistance to the late blight oomycete and their tubers were protected in storage from infection by the soft rot-causing bacteria. In other work, raspberry plants engineered with the gene coding for the common plant polygalacturonase-inhibiting protein (PGIP) became resistant to the gray mold fungus *Botrytis cinerea*, although the transgene in raspberry, but not in other

FIGURE 1-40 Increased resistance to disease through biotechnology. Comparison of "Sunrise" papaya plants susceptible to *papaya ringspot virus* (PRSV) surrounding a block of the genetically similar "Rainbow" papaya plants that had been transformed for resistance to PRSV. Both "Sunrise" and transgenic "Rainbow" plants were inoculated by natural PRSV inoculum. (A) "Sunrise" (left) and transgenic "Rainbow" (right) plants 9 (B) 18, and (C) 23 months after transplanting. (D) Aerial photograph of the "Rainbow" block 28 months after transplanting, by which time the "Sunrise" plants surrounding the "Rainbow" block are almost totally destroyed by the virus, whereas the transgenic "Rainbow" plants remained free of virus, look healthy, and produced excellent yields. [Photographs courtesy of Ferreira (2002). *Plant Dis.* **86**, 101–105.]

plants, is expressed only in immature green fruit.

In addition to helping us engineer plants resistant to disease, molecular biology and biotechnology have made possible the development and use of nontoxic chemical substances that, when applied to plants externally, stimulate the plants and elicit the activation of their natural defense mechanisms, i.e., activation of the localized defense mechanism (hypersensitive response) and systemic-aquired resistance (SAR). Two such chemical substances that have been proven effective and are used commercially are Actigard, where one application increases the plants' resistance against some bacterial and some fungal diseases for several weeks, and Messenger, derived from the fire blight bacterium gene coding for the protein harpin, which elicits a hypersensitive response and SAR in plants. Messenger, which also promotes plant growth, is effective against a variety of diseases of several crops, including strawberry, tomato, and cotton.

In transforming plants for disease resistance or for any other characteristic, it is necessary to modify their nucleic acid by adding genetic material from another plant or, rarely, from an animal or a pathogen. In most cases, these nucleic acids are or become active, producing in the plant compounds that may be toxic to pathogens or pests and, possibly, to humans. In addition, some of this nucleic acid may find its way, through cross-pollination or through transfer by microorganisms, into weeds or other wild plants, making these plants also resistant to the pathogen or pest. Several kinds of plants have been engineered to produce toxins against certain insects; to produce vaccines against certain human pathogens; to produce animal or human growth hormones; or to produce pharmaceutical compounds

continued

that can be used to treat diseases of humans and animals. The fear by some people that some or all of these products will get into the human diet or in the animal food chain and cause allergies and other adverse health effects has resulted in significant unfavorable publicity for such products and for biotechnology. That type of publicity has, in turn, led many large buyers to refuse to buy and use products produced by genetically modified organisms (GMO). Following the adverse publicity, several governments, especially in Europe, passed laws and raised barriers to the importation of products derived from genetically modified organisms.

In addition to the argument against introducing into crops, through genetic engineering, new proteins that may cause allergic reactions in some people, there have also been arguments against biotechnology because it takes possession of, patents, and monopolizes genetic material that was previously available and free to everybody; it replaces the numerous sustainable local varieties with a few genetically engineered ones, the seed of which the farmers must buy from large companies every year; it threatens the development of pests and pathogens that can resist or overcome the transformed resistant crops; it threatens to lead to the use of larger amounts of herbicides with crops like those made herbicide resistant while the weeds are still susceptible; it threatens unknown numbers of nontarget organisms that may be affected adversely by the protein; it threatens to upset the plant balance, and through it the entire biotic balance of the environment, by having such new genes transferred naturally to nontarget plants and their proteins, harmless or not, consumed by microorganisms, animals, and humans unaccustomed to such proteins; it threatens the occurrence of accidents in which crops transformed for the production of pharmaceuticals, vaccines, and so on become mixed with edible crops.

BOX 13 Food safety

In recent years, food safety has been threatened by a number of events and developments that allow foodborne microorganisms pathogenic to humans, e.g., the bacteria *Salmonella*, *Listeria*, *Escherichia coli* strain *0157:h17*, the protozoa *Cyclospora*, *Cryptosporidium*, and *Giardia*, and the *hepatitis A virus*, to reach and contaminate our food in a variety of ways. These include (a) increased processing of fresh plant produce (e.g., fruit juices, fruit or vegetable purees, cole slaw, fruit sections and cut-up vegetables for salads in bulk or in plastic bags) that may sometimes contain produce that carries a significant amount of food-spoiling bacteria and mycotoxin-producing fungi; (b) inadequate food processing procedures that allow survival of human pathogens in the processed product; (c) long storage of foods that encourages the development of pathogenic microorganisms; (d) application to fruit and vegetable fields of improperly aged or poorly treated manure that carries human pathogens; (e) application on the plants of irrigation water that may be carrying one or many of the aforementioned human pathogens due to contamination by humans and animals through run-off of waste waters, etc.; (f) unacceptable hygiene of harvesters, handlers, and packers after using the toilet that results in the contamination of fruits and vegetables with human pathogens; and (g) the presence of pets, livestock, and wildlife animals, some of which may carry human pathogens on their bodies or in their feces to fruits and vegetables. To these should be added the ever-increasing shipment of food items among various geographical points of a country and worldwide, which may greatly multiply and expand the effects of a local contamination of food products.

One of the main effects of fears about food safety is economic. Not only is it costly to take all measures necessary to secure food safety, but there is also the fear and cost of rejection of produce shipments at the point of destination. Similarly, there is the possibility of refusal of buyers to purchase produce from farms that do not meet the buyer's food safety standards. In the United States and other developed countries, many of the large buyers of food products for their mills, processing factories, or chain stores demand third-party audits of farms by certified specially trained individuals and consulting firms regarding the employment by the farm of all necessary precautions in the type of manure they may be using, the quality of water used for irrigation, the health and hygiene of their workers and plant handlers, and so on. Also, to avoid unjustified accusations of offering contaminated produce, farmers are or will soon be expected to have a trace-back system in place. This will happen by identifying all produce leaving the farm as to origin and date of packing so that if contamination is found in the produce in the marketplace, the source will be easy to identify and appropriate measures may be taken. Also, it will become necessary to keep food safety records, such as documenting worker training sessions, recording the results of water tests, details of manure applications, if any, of dates, methods, and rates of irrigation, and so on, as well as of disease outbreaks among the farm workers. To protect themselves from purchasing contaminated produce, buyers of large quantities will test or have the produce tested with serological and molecular-based diagnostic techniques that can already detect, for example, as few as three *Salmonella* cells per 25 grams of naturally contaminated food.

In addition to the aforementioned types of contamination of food with pathogens, there are the additional threats of contamination with pathogenic microorganisms that are resistant to antibiotics, such as streptomycin and tetracyclines used in plants, as well as in humans and animals; the presence in the food of genetically engineered plants that contain genes for chemicals toxic to insects, such as the *Bacillus thuringiensis* toxin; genes for antibiotics against other human pathogens; genes for activating defensive mechanisms of plants,

often through the production of proteins and phenolic compounds that make the plants resistant to insects, diseases, and to herbicides; genes for edible or otherwise delivered human vaccines and antibodies (plantibodies) against human pathogens; genes for unrelated proteins that may be allergenic in some individuals; and even genes for producing plastic. There is fear in some segments of the population, especially in developed countries, that although some of these genes are introduced into inedible plants such as tobacco, plants with such genes will intentionally or accidentally find their way into foods and feed and will affect adversely the health of animals and humans. Many large produce distributors or retailing companies and manufacturers of food products simply refuse to buy any produce that comes from genetically modified organisms (GMOs), plants, or animals. Molecular-based diagnostic tests have also been developed that detect introduced genes that may not have been declared as being present.

Since the horrendous terrorist attack in New York and Washington, DC, in September of 2001 and the subsequently declared war against terrorists wherever they exist, there is an added fear of having food contaminated intentionally by terrorists. Contamination could be carried out with human pathogenic microorganisms, such as those mentioned earlier or with others, e.g., the bacterium causing the disease anthrax, or with toxic substances. Contamination of produce can be done while the latter is still in the field, in transit, or in grocery stores. There is also fear of having the drinking water or the water used for irrigation of fruits and vegetables contaminated intentionally by terrorists with pathogenic microorganisms or with toxic substances that will then find their way to humans via the food distribution system. This subject is discussed further in the following section.

BOX 14 Bioterrorism, agroterrorism, biological warfare, etc. who, what, why?

Bioterrorism is loosely defined here as the use, or threat of use, of biological agents, mainly pathogenic microorganisms that could infect people and cause disease and, thereby, instil fear and terror in all of the populace. Bioterrorism may differ from biological warfare in that the latter is usually directed against enemy armies and its purpose is to incapacitate or kill enemy soldiers, whereas in bioterrorism the purpose is to frighten and terrorize civilian populations, although casualties in large numbers may or may not occur. The most vivid example of bioterrorism occurred in the fall of 2001 when persons in various positions in politics and the television news media in New York and Washington received letters through the mail containing spores of the bacterium *Bacillus anthracis*, the cause of the severe and often deadly anthrax disease. It became apparent at the time that the perpetrators of the anthrax bioterrorism, or others, could easily expand to other forms of bioterrorism by either contaminating agricultural products such as vegetables, milk, or meat on the farm or in the store with microorganisms pathogenic to humans, which would scare buyers away from such products (agroterrorism), or by spreading selected plant pathogenic microorganisms on certain crops, e.g., cereals, potatoes, and corn, which they could infect and destroy to various extents, thereby causing devastating losses that would further increase the fear of the people.

Biological warfare has been talked about for several decades and many of the larger countries have been producing and stockpiling pathogenic microorganisms, such as the anthrax bacterium, for potential use against the army of an enemy country with which they might go to war. At the same time, however, several countries have been experimenting with and stockpiling microorganisms that can infect and destroy important staple food crops for certain countries, e.g., rice, potatoes, wheat, or beans, which could affect the availability of food and thereby survival of the people, or at least, their will to fight and prolong the war. This type of agricultural biological warfare has revolved around important pathogens of such crops, e.g., *Magnaporthe grisea*, the fungus causing the blast disease of rice; *Phytophthora infestans*, the oomycete causing the late blight of potato; and *Puccinia graminis*, the fungus causing the rust diseases of wheat and other small grains.

As the specialization of crops in each area increases and as our knowledge of diseases of such crops increases, it becomes evident that such areas or countries become extremely vulnerable to agroterrorism or agrosabotage. This happens even if, or especially if, they grow relatively small areas of such specialty crops, e.g., bananas, citrus, coffee, and cacao, which are the main export crop and the main source of foreign currency for these countries. For each area producing such a crop there are pathogens of the crop elsewhere that, if introduced, could destroy the crop for the year to come and, possibly, forever. The pathogens that would be used on such clonal, genetically uniform, perennial crops are likely to be insect-vectored bacteria, phytoplasmas, or viruses. Such pathogens can be introduced into a field as a few bacteria- or virus-carrying insect vectors that would feed on and infect some of the plants and then, in the same or in subsequent years, multiply and spread the pathogen they carry to more plants over a continually expanding area.

WORLDWIDE DEVELOPMENT OF PLANT PATHOLOGY AS A PROFESSION

As mentioned earlier, plant pathology had its origins in plant pathological observations and studies made by botanists, naturalists, and physicians in Europe in the mid- to late 1800s. Soon after, plant pathological activity shifted primarily to the United States, where it has remained at a high level to date.

The students of the first, self-made, plant pathologists began to be hired as plant pathologists by state agricultural experiment stations, by the federal Department of Agriculture, and by universities at which they taught courses in plant pathology. In 1891, the plant pathologists in the Netherlands formed the Netherlands Society of Plant Pathology and began publishing the *Netherlands Journal of Plant Pathology* in 1895. In 1908, the plant pathologists in the United States organized into the American Phytopathological Society, and they too decided to publish a journal of plant pathology in which they could present the results of their own research and could read about the work of their colleagues. The journal, named *Phytopathology*, began to be published in 1911 as an international journal of plant pathology. The Phytopathological Society of Japan was founded in 1916, and its journal began to be published in 1918. In subsequent decades, plant pathologists formed associations and began publishing plant pathological journals in several other countries, e.g., Canada (1930) and India (1947). In the second half of the 20th century, plant pathologists in nearly 50 more countries organized into professional associations; some of them, as in Brazil, published their own national journals, whereas others formed multinational unions, e.g., the Latin American Phytopathological Association, or published a regional journal such as *Phytopathologia Mediterranea*. In 1968, an International Society of Plant Pathology was formed and it held the first International Congress of Plant Pathology in London that same year. By the end of the 20th century most or all countries have one or more plant pathologists, although in many developing countries that person is an administrator of some kind or a professor at a university. Nevertheless, in many parts of the world, plant pathology is generally unknown or rarely practiced, and losses from plant diseases in developing countries are still great.

International Centers for Agricultural Research

In the mid-1940s, the Rockefeller Foundation, in cooperation with the Mexican government, established a program in Mexico for interdisciplinary research on basic food crops such as wheat, corn, potatoes, and beans. That program was so successful in improving crops and in training personnel in the technologies that similar Rockefeller Foundation programs were established in Colombia, Chile, and India. It soon became apparent, however, that it would not be possible to have such programs in every developing country; rather, it would be preferable to have a few international centers concentrating on one or a few basic crops. So, with the cooperation of the local governments and funding from the Rockefeller and the Ford foundations, the International Rice Research Institute (IRRI) was established in the Philippines in 1960, the International Maize and Wheat Improvement Center (CIMMYT) in Mexico in 1966, the International Institute of Tropical Agriculture (IITA) in Nigeria in 1968, and the International Center of Tropical Agriculture (CIAT) in Colombia in 1969 (Fig. 1-41).

The success of these centers suggested the need for additional ones. As the finances required to operate the earlier and the new centers were beyond the means of the Ford and the Rockefeller foundations, they, in collaboration with the World Bank, set up a consortium of potential donors interested in financing international agricultural research. The consortium, known as the Consultative Group on International Agricultural Research (CGIAR), consists of wealthy countries, development banks, and other foundations and agencies. The CGIAR receives help in determining research priorities from a technical advisory committee, which consists of 13 scientists and economists. Additional centers established by the consultative group include the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in India in 1972 and the International Potato Center (CIP) in Peru, also in 1972. A similarly operating center but not funded by the consultative group, namely the Asian Vegetable Research and Development Center (AVRDC) in Taiwan, was also established in 1972. More recent centers include the International Center for Agricultural Research in the Dry Areas (ICARDA) in Syria, the West Africa Rice Development Association (WARDA) in Gold Coast, and some others (Fig. 1-41): IFPRI, International Food Policy Research Institute; ISNAR, International Service for National Agricultural Research; IPGRI, International Plant Genetic Resources Institute; ILRI, International Livestock Research Institute; ICRAF, International Center for Research in Agroforestry; IIMI, International Irrigation Management Institute; CIFOR, Center for International Forestry Research; and ICLARM, International Center for Living Aquatic Resources Management.

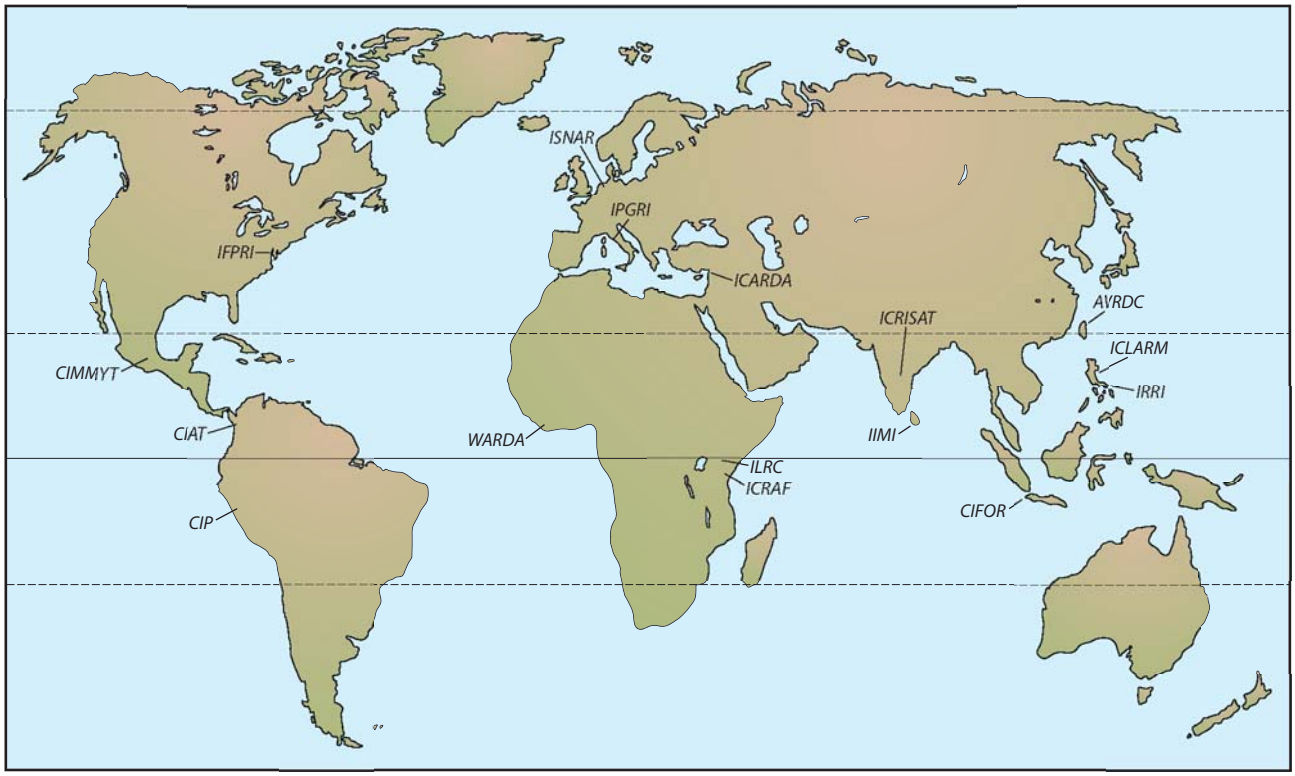

FIGURE 1-41 The global agricultural research system.

Each of the aforementioned centers studying plants includes several plant pathologists working on diseases of the specific crop(s) studied by the center. The contributions of the resident plant pathologists to the study of these diseases and to the development of disease-resistant cultivars and other controls against the diseases of these crops have been truly great. These pathologists have also helped train many other scientists not only of the host country, but from many other developing countries attempting to grow these crops, have taught plant pathology courses in universities with which their center is affiliated, and have generally helped to significantly reduce losses of crops caused by plant diseases.

The need for plant pathology has always been particularly great in tropical countries primarily because the tropical climate (hot and usually humid) favors the survival and multiplication of pathogens throughout the year, as well as the prolonged or continuous presence of primary and alternate hosts and large numbers of active vectors such as insects. Tropical climates also favor multiple and continuous infections by pathogens, which often lead to devastating epidemics. These problems in tropical countries are further compounded by low educational levels and lack of funds for carrying out effective plant disease control programs. Moreover, tremendous losses from disease occur in the tropics in all types of produce after harvest because many harvested products are already infected or contaminated while still in the field and also because harvested products often rot in storage or transit due to lack of appropriate decontamination and lack of any kind of refrigeration. It is not surprising, therefore, that so many of the international centers for agricultural research have been established in the tropics, nor that their contributions have had a big and immediate impact on reducing losses from disease. Much more, however, remains to be done.

Trends in Teaching and Training in Plant Pathology

The first course in plant pathology was offered at Harvard University by M. A. Farlow in 1875. In the early 1900s, departments of plant pathology began to be established at some of the larger universities, often as departments of botany and plant pathology. The early courses were, by necessity, primarily descriptive of the diseases of various types of crops (vegetables, fruit trees, field crops), in addition to providing information on

the development of some of the pathogens and diseases and on possible control measures. General textbooks in plant pathology appeared in several languages. In the United States the main textbooks were those by Duggar (1906), Stevens and Hall (1921), Heald (1926, 1943), and Walker (1950). In the meantime, specialized books were published on plant pathogenic fungi and, later on, bacteria, viruses, and nematodes and the diseases they cause, as well as on all types of diseases of groups of crops, such as vegetables, field crops, and fruit crops. Starting in the 1960s, more specialized books on the physiology, biochemistry, epidemiology, and genetics of plant diseases were published.

Students training to become plant pathologists took as many relevant courses as were available at their university, but they learned most of their trade by watching and working together with their mentor–professor plant pathologist and by themselves, under some supervision, doing research on a specific plant disease or pathogen. Such studies, when successful, eventually earned them a doctor of philosophy (Ph.D.) degree in plant pathology, which indicates that they have the ability, knowledge, and training to do research, i.e., to solve scientific, and possibly practical, problems in plant pathology. This type of training continues to date except that, because of the tremendous increase in the amount of knowledge in plant pathology, students specialize a great deal more in what they learn and do. This has been particularly evident in the years after 1985 during which molecular plant pathology has attracted many of the students working toward their Ph.D. in plant pathology. Most of the holders of a Ph.D. in plant pathology find jobs as professors in colleges or universities, or as researchers in universities, government, or industry. Some develop their own business as private practitioners or consultants to growers. A few, usually one or two per state, work as extension plant pathologists in state land grant universities and experiment stations, where they are responsible for transferring plant pathology information from plant pathology researchers to growers and county agents, visiting crop fields and identifying diseases, identifying diseases in plant samples sent in by growers, and developing and disseminating disease control recommendations.

Similar but less extensive and intensive course work and research training can lead to a master of science (M.S.) degree in plant pathology. This enables the holder to work for the same agencies as the Ph.D. holders but with reduced responsibility and benefits. Several departments of plant pathology also offer bachelor of science (B.S.) degrees in plant pathology, which serve either as intermediate steps for advanced degrees or enable the holders to work in university, government, and industry laboratories, for various types of agribusinesses as chemical, seed, etc., company representatives, or as private practitioners.

Plant pathology, unlike its sister sciences of medicine and veterinary medicine, deals with plant diseases caused by pathogens and, to some extent, by environmental factors. It does not have teaching and training programs that will produce practitioners similar to the general practitioner physicians and veterinarians, i.e., professionals capable of identifying all types of causes of disease and injury to plants and of making recommendations to control or manage these. Such practitioners (plant doctors) would also be trained in identifying and making control recommendations for insects, weeds, damage by animal wildlife, and the nutritional and other environmental conditions that affect plant health. Development of a program leading to a professional doctor of plant medicine or doctor of plant health degree, similar to the M.D. (doctor of medicine) and D.V.M. (doctor of veterinary medicine) degrees, had been discussed since the late 1980s and was offered for the first time by the College of Agriculture and Life Sciences of the University of Florida in the year 2000.

Plant Disease Clinics

For many years, most states operated a plant disease clinic through their department of plant pathology. Growers, county extension agents, and home owners would send diseased plants, soil from areas with diseased plants, and sometimes insects to the plant disease clinic and the pathogen or insect would be identified and control measures would be recommended, all free of charge. At first, the plant disease clinics were set up rather informally and were supervised by the extension plant pathologist, with most of the diagnoses made by advanced plant pathology graduate students assisted significantly by more junior graduate students. Early plant disease clinics were equipped primarily with surface sterilants, dissecting scopes, microscopes, culture dishes and test tubes, and nutrient media for culturing fungi and bacteria. Later, much of the day-to-day operation of plant disease clinics was turned over to M.S. or Ph.D. plant pathologists hired specifically for that purpose. At the same time, nematode isolation from roots or soil and plant nematode identification became integral functions of the plant disease clinics. Virus disease identification was still made by host symptomatology alone, but some host range tests for diagnostic purposes were carried out.

Since the 1970s, every state has at least one plant disease clinic and some have several; e.g., Florida has four plant disease clinics. In addition to state-funded

plant disease clinics, in some states there may be one or more privately run plant disease clinics and, in a few states, a plant disease clinic may also be operated by the state department of agriculture. Today's plant disease clinics often have one scientist with an advanced degree and one or more laboratory assistants; they are also equipped for viral disease diagnosis through host range tests, serological tests, cell inclusion identification, electron microscopy of plant sap, and dot-blot assays of radioactive or color-producing DNA probes. Plant disease clinics also have modern computers with databases and expert systems for disease and pathogen identification, computerized distance diagnostic systems that transmit plant disease images directly from the field to an expert diagnostician, CD videodisc capabilities, and e-mail for transmitting the results of diagnosis and the recommendations for control to their clientele. Also, however, due to increased costs for these tests and services, plant disease clinics in many states have now established fees that must be paid by all commercial users and home owners submitting samples of diseased plants for diagnosis.

The Practice and Practitioners of Plant Pathology

The science of plant pathology has been and continues to be developed primarily by highly specialized professors or researchers who have advanced, usually doctorate, degrees. For many discoveries, considerable contributions are made by graduate students who are themselves working toward M.S. or Ph.D. degrees at departments of plant pathology, botany, or biology and at agricultural experiment stations.

The practice of plant pathology, however, is carried out at a much lower scientific and professional level. Medicine and veterinary medicine also have Ph.D.-holding scientists who do research. These scientists advance the respective sciences at various universities and research centers. In addition, however, both medicine and veterinary medicine have numerous highly trained practicing physicians (doctors of medicine) and veterinarians (doctors of veterinary medicine) who are the practitioners of each science. They diagnose the ailments and prescribe treatments for humans and animals, both individuals and populations. In contrast, plant pathology has few well-trained practicing plant pathologists.

In general, most states have one or two extension plant pathologists. Their duty is to (a) transfer the information developed by the researchers in the state and elsewhere to county extension personnel and to growers and (b) demonstrate its effectiveness to those who need it, i.e., the growers. The same extension plant pathologists are expected to be able to diagnose all diseases on all types of plants, regardless of their cause, and to recommend measures for their control. The extension plant pathologists also train the county extension agents, who usually have little formal education or training in plant pathology, so that they can diagnose and offer recommendations for the control of plant diseases common in their county. Many states have a plant disease clinic to which samples of diseased plants or plant parts are sent by growers, home owners, and county agents for diagnosis and control recommendations. In some of the most agriculturally oriented states, a few persons, who usually have varying levels of education and training in plant pathology (B.S., M.S., or Ph.D.), offer their services as private practitioners (plant doctors) to individual growers or groups of growers, or they operate their own private plant disease clinics. Much of the time, however, growers receive information on plant diseases and recommendations for plant disease control from salesmen of pesticides, seeds, or fertilizers, and from other professionals (agronomists, horticulturists, entomologists, etc.) who may have little or no education and training in plant pathology.

Under the present conditions, therefore, most growers often receive rather limited, delayed, or inaccurate information on the kinds and development of diseases affecting their crops and, similarly, incomplete and sometimes inaccurate information about their control. As a result, plant diseases are often detected late and are sometimes misdiagnosed, and frequently the wrong pesticides or excessive dosages of pesticides are recommended and applied for their control. The amount of crop losses to plant diseases, therefore, and possibly contamination of the environment with pesticides as well, is often greater than need be.

Certification of Professional Plant Pathologists

When a professional such as a physician, veterinarian, lawyer, or engineer offers his or her services to individuals, the individuals expect the professional to have appropriate education and training that meet or exceed certain professional and ethical standards. At the same time, the professional and the public also expect that no person who does not meet such a standard will be allowed to provide such services: the professionals because they do not want such persons to compete for business with them and the public because they want to be certain that the person to whom they go for such services can actually provide them correctly. These two expectations are generally guaranteed through the licensing programs operated by each state and country.

Since the 1960s and 1970s, many states have required the licensing of pest control advisers, pesticide applicators, etc. In addition, several professional societies, such as the American Society of Agronomy, the Soil Science Society of America, the Crop Science Society of America, and the Entomological Society of America, have established professional certification programs that resulted in certified agronomists, certified soil scientists, certified crop scientists, certified entomologists, and so on.

A proposal for establishing an American registry of professional plant pathologists was submitted to the American Phytopathological Society in 1980, but it was not approved until 1991. The following year, a certified professional plant pathologist program was developed that set professional and ethical standards. A board of six plant pathologists, named by the American Phytopathological Society, was authorized to review and compare the credentials (course work, experience, references) of each applicant with the standard and to determine their eligibility to become certified professional plant pathologists. Because there were already many practicing plant pathologists (private consultants) when the certification program came into being, the standards for certification were set so that it would include most of them. The standards include a B.S. degree in plant pathology and 5 years of professional experience, a M.S. in plant pathology and 3 years of professional experience; or a Ph.D. in plant pathology and 1 year of professional experience. The board also set a curriculum that would enable new students to become certified professional plant pathologists. In addition, the board set standards for continual education and training so that certified professional plant pathologists can keep abreast of new information, techniques, conditions, regulations, and requirements in the area of plant health management.

BOX 15 Plant pathology as a part of plant medicine: the doctor of plant medicine program

In the last two decades, considerable efforts have been made to broaden the concepts of both plant health and plant protection. The American Phytopathological Society, realizing the need for such a broader concept, launched a new electronic journal called "Plant Health Progress," which publishes articles on all facets of plant health.

It has become apparent, however, that trained professionals are needed who can deal with the whole health of the plant and give recommendations for its maintenance or restoration. Such professionals would be able to diagnose all causes of plant problems, be they pathogens (fungi, bacteria, viruses, nematodes, parasitic algae and parasitic higher plants, protozoa, etc.), insects, mites, vertebrate (birds, field mice, deer) and invertebrate (snails, slugs) wildlife, weeds, soil conditions, weather extremes, pollutants, and so on, and to recommend strategies for their management or control. To develop such a broad expertise in plant protection, however, it is necessary that qualified graduates in a biological or agricultural science attend a 3- to 4-year profes-sional graduate degree program. The University of Florida's College of Agriculture and Life Sciences created such a program in 1999 and accepted its first graduate students in the fall semester of 2000. The Doctor of Plant Medicine (DPM) program, as it is called, had 14 students the first year, 15 the second year, and 10–14 students per year thereafter.

The degree is called Doctor of Plant Medicine rather than Doctor of Plant Health because it parallels the other two doctorates in the health professions, those of medicine (MD) and of veterinary medicine (DVM), in so many aspects that its goals and functions are easier to understand by this name. In addition, just like the MD and the DVM, the DPM is a professional, practitioner's degree, not a research degree as is the Ph.D. None of these degrees (MD, DVM, DPM) are replacing the Ph.D.s in their respective areas. Instead they provide a mechanism by which the information generated by the researcher Ph.D.s is used for the corresponding clientele (humans, animals, plants), the ailments of which are diagnosed and managed or controlled. Also, just like MD and DVM students, DPM students do several projects that involve mainly applied-type research and write appropriate reports, but they do not do research on a single project and do not write a thesis or dissertation.

The DPM program accepts students who have graduated with a bachelor's or a master's degree, preferably, but not necessarily, in a biological or agricultural discipline. Entering students must meet all criteria other graduate students (for Ph.D. or M.S. degrees) must meet. DPM students take 90 credits of graduate courses in the appropriate academic departments, most courses with laboratories, generally being the same courses taken by the graduate students of each department or discipline. About 65 of these credits are in required courses, a minimum of 18 in plant science, including courses in crop production, soils and crop nutrition, and weed science, 17 in entomology, 18 in plant pathology, 5 in nematology, 2 in acarology, 2 in wildlife that damage plants, 5 in plant pest management, and courses in agribusiness management, marketing, and agricultural law. The elective credits may be used by the student to specialize in a commodity area of his/her choice (e.g., agronomic crops, horticultural crops, ornamental crops and/or turf, forestry and/or urban forestry, education courses for college teaching, etc.).

In addition to the 90 credits of courses, DPM students must also do 30 credits of internships or practicums by

spending appropriate lengths of time (2–3 credits each) in the soil analysis laboratory, the plant disease clinic, the nematode assay laboratory, the insect identification laboratory, and the weed identification laboratory. The students may also elect to do internships by working side by side with the extension weed scientist, horticulturist, plant pathologist, or entomologist, or they may elect to do an internship at an agricultural experiment station, at an agrichemical or seed company, or working side by side with an experienced crop consultant. The location of internships may vary from local to international. The entire curriculum is expected, although not required, to be completed within 3 or 4 years. Part-time students may take considerably longer.

Upon completion of the program, DPM graduates receive the doctorate degree and are fully educated and trained plant doctors who can identify just about anything, living and nonliving, that causes damage to plants and can provide quick and correct recommendations for their management or control. Their education, training, expertise, and the doctorate degree qualify them for a variety of well-paying jobs within the United States and internationally, including private practitioners as crop consultants; working for large farms or agribusinesses; working for the state or federal extension service (as county agents, IPM coordinators, pesticide information coordinators, etc.), for state or federal regulatory agencies [e.g., the Animal and Plant Health Inspection Service (APHIS), the Plant Protection and Quarantine (PPQ) Service, ship and airport inspectors, etc.); working for agrichemical, seed, and large food companies such as Del Monte and Campbell, teaching various biological courses at 2- and 4-year colleges and universities; and working for mid- to large size municipalities.

PLANT PATHOLOGY'S CONTRIBUTION TO CROPS AND SOCIETY

Some Historical and Present Examples of Losses Caused by Plant Diseases

Plant diseases affect the existence, adequate growth, and productivity of all kinds of plants and thereby affect one or more of the basic prerequisites for a healthy, safe life for humans. This happened since the time humans gave up their dependence on wild game and fruits and became more stationary, domesticated, and began to practice agriculture more than 6000 years ago. Destruction of food and feed crops by diseases has been an all too common occurrence in the past. It has resulted in malnutrition, starvation, migration, and death of people and animals on numerous occasions, several of which are well documented in history. Similar effects are observed annually in developing agrarian societies in which families and nations are dependent for their sustenance on their own produce. In more developed societies, losses from diseases in food and feed produce result primarily in financial losses and higher prices. It should be kept in mind, however, that loss of any amount of food or feed because of plant diseases means there is less available in the world economy. Considering the chronically inadequate amounts and distribution of food available, rich people and rich countries will be able to acquire such foodstuffs from wherever they are available, whereas many poor people somewhere in the world will be worse off because of these losses, and will go hungry.

Some examples of plant diseases that have caused severe losses in the past are shown in Tables 1-2 and 1-3.

Plant Diseases and World Crop Production

There are no dependable surveys of numbers of humans living on the earth before the year 1900. It is estimated, however, that there were about 300 million people living on the earth in the year A.D. 1, 310 million in A.D. 1000, 400 million in A.D. 1500, and 1.3 billion in A.D. 1900. During the 20th century there has been a dramatic explosion in the human population. Despite recent efforts to reduce the rate of population growth, the number of new humans added to the world population each year and the additional demands for food, energy, and other resources from our planet are frightening. Thus, the world population in 1993 was about 5.57 billion, and, at the present rate of 1.7% annual growth, it was expected to be 6.2 billion by the year 2000, be 7.1 billion by the year 2010, and be 8.5 billion by 2025. Currently, the world population increases by 1 billion every 11 years (see Fig. 1-42).

Paradoxically, the developing countries, in which from 50 to 80% of the population is engaged in agriculture, have the lowest agricultural output, their people are living on a substandard diet, and they have the highest population growth rates (2.64%). Because of the current distribution of usable land and population, of educational and technical levels for food production, and of general world economics, it is estimated that even today some 2 billion people suffer from hunger, malnutrition, or both. To feed these people and the additional millions to come in the next few years, all possible methods of increasing the world food supply are currently being pursued, including (1) expansion of crop acreages, (2) improved methods of cultivation, (3) increased fertilization, (4) use of improved varieties

TABLE 1-2
Examples of Severe Losses Caused by Plant Diseases

Disease	Location	Comments
Fungal		
1. Cereal rusts	Worldwide	Frequent severe epidemics; huge annual losses
2. Cereal smuts	Worldwide	Continuous, although lesser, losses on all grains
3. Ergot of rye and wheat	Worldwide	Infrequent, poisonous to humans and animals
4. Late blight of potato	Cool, humid climates	Annual epidemics, e.g., Irish famine (1845–1846)
5. Brown spot of rice	Asia	Epidemics, e.g., the great Bengal famine (1943)
6. Southern corn leaf blight	U.S.	Historical interest, epidemic 1970, $1 billion lost
7. Powdery mildew of grapes	Worldwide	European epidemics (1840s–1850s)
8. Downy mildew of grapes	U.S., Europe	European epidemic (1870s–1880s)
9. Downy mildew of tobacco	U.S., Europe	European epidemic (1950s–1960s); epidemic in North America (1979)
10. Chestnut blight	U.S.	Destroyed almost all American chestnut trees (1904–1940)
11. Dutch elm disease	U.S., Europe	Destroying American elm trees (1918 to present)
12. Pine stem rusts	Worldwide	Causing severe losses in many areas
13. Dwarf mistletoes	Worldwide	Serious losses in many areas
14. Coffee rust	Asia, South America	Destroyed all coffee in southeast Asia (1870s–1880s) since 1970 present in South and Central America
15. Banana leaf spot or Sigatoka disease	Worldwide	Great annual losses
16. Rubber leaf blight	South America	Destroys rubber tree plantations
17. Fusarium scab of wheat	North America	Severe losses in wet years
Viral		
18. Sugar cane mosaic	Worldwide	Great losses on sugar cane and corn
19. Sugar beet yellows	Worldwide	Great losses every year
20. Citrus tristeza (quick decline)	Africa, Americas	Millions of trees being killed
21. Swollen shoot of cacao	Africa	Continuous heavy losses
22. Plum pox or sharka	Europe, North America	Spreading severe epidemic on plums, peaches, apricots
23. Barley yellow dwarf	Worldwide	Important on small grains worldwide
24. Tomato yellow leaf curl	Mediterranean countries, Caribbean Basin, U.S.	Severe losses of tomatoes, beans, etc.
25. Tomato spotted wilt virus	Worldwide	On tomato, tobacco, peanuts, ornamentals, etc.
Bacterial		
26. Citrus canker	Asia, Africa, Brazil, U.S.	Caused eradication of millions of trees in Florida in 1910s and again in the 1980s and 1990s
27. Fire blight of pome fruits	North America, Europe	Kills numerous trees annually
28. Soft rot of vegetables	Worldwide	Huge losses of fleshy vegetables
Phytoplasmal		
29. Peach yellows	Eastern U.S., Russia	Historical, 10 million peach trees killed
30. Pear decline	Pacific coast states and Canada (1960s), Europe	Millions of pear trees killed
Nematode diseases		
31. Root knot	Worldwide	Continuous losses on vegetables and most other plants
32. Sugar beet cyst nematode	Northern Europe, Western U.S.	Continuous severe annual losses on sugar beets
33. Soybean cyst nematode	Asia, North and South America	Continuous serious losses on soybean

of crops, (5) increased irrigation, and (6) improved crop protection.

Crop Losses to Diseases, Insects, and Weeds

There is no doubt that the first five of the aforementioned measures must provide the larger amounts of food needed. Crop protection from pests and diseases can only reduce the amount lost after the potential for increased food production has been attained by proper utilization of all means possible. Crop protection, of course, has been important in the past and is important now. For example, it is estimated that in the Untied States alone, despite the control measures practiced, each year, crops worth $9.1 billion are lost to diseases,

TABLE 1-3
Additional Diseases Likely to Cause Severe Losses in the Future

Disease	Comments
Fungal	
1. Late blight of potato and tomato	New mating type of fungus spreading worldwide
2. Downy mildew of corn and sorghum	Just spreading beyond southeast Asia
3. Karnal bunt of wheat	Destructive in Pakistan, India, Nepal; since the 1980s introduced into Mexico and in the 1990s into U.S.
4. Soybean rust	Spreading from southeast Asia and from Russia; already in Hawaii, Puerto Rico, and South America
5. Monilia pod rot of cacao	Very destructive in South America; spreading elsewhere
6. Chrysanthemum white rust	Important in Europe, Asia, and recently in California
7. Sugar cane rust	Destructive in the Americas and elsewhere
8. Citrus black spot	Severe in Central and South America
9. Sweet orange scab	Severe in Australia
Viral	
10. African cassava mosaic	Destructive in Africa; threatening Asia and the Americas
11. Streak disease of maize (corn)	Spread throughout Africa on sugar cane, corn, wheat, etc.
12. Hoja blanca (white tip) of rice	Destructive in the Americas so far
13. Bunchy top of banana	Destructive in Asia, Australia, Egypt, Pacific islands
14. Rice tungro disease	Destructive in southeast Asia
15. Bean golden mosaic	Caribbean basin, Central America, Florida
16. Tomato yellow leaf curl.	East Mediterranean, Caribbean, the Americas
17. Plum pox	Destructive in Europe, spreading into U.S.
Bacterial	
18. Bacterial leaf blight of rice	Destructive in Japan and India; spreading
19. Bacterial wilt of banana	Destructive in the Americas; spreading elsewhere
20. Pierce's disease of grape	Deadly in southeast U.S.; spreading into California
21. Citrus variegation chlorosis	Destructive in Brazil; spreading
22. Citrus greening disease	Severe in Asia; spreading
Phytoplasmal	
23. Lethal yellowing of coconut palms	Destructive in Central America; spreading into U.S.
Viroid	
24. Cadang-cadang disease of coconut	Killed more than 15 million trees in the Philippines to date
Nematode	
25. Burrowing nematode	Severe on banana in many areas and citrus in Florida
26. Red ring of palms	Severe in Central America and the Caribbean
27. Pinewood nematode	Widespread and becoming severe in North America

$7.7 billion to insects, and $6.2 billion to weeds. Crop protection, however, becomes even more important in an intensive agriculture, where increased fertilization, genetically uniform high-yielding varieties, increased irrigation, and other methods are used. Crop losses to diseases and pests not only affect national and world food supplies and economies but also affect individual farmers even more, whether they grow the crop for direct consumption or for sale. Because operating expenditures for the production of the crop remain the same in years of low or high disease incidence, harvests lost to disease and pests lower the net return directly.

The amount of each crop lost to pests varies with the crop (e.g., 23.4% for fruits, 34.5% for cereals, 55.0% for sugar cane). Crop loss varies with the type of climate (warm, humid, rainy, dry, etc.), the particular year, availability of pesticides, availability of trained personnel, and educational level of growers. Also, the importance of each kind of pest (diseases, insects, weeds) varies with the crop. Generally, diseases, which are more difficult to detect, identify, and control on time, cause losses of about 14% of the crop; insects, if left unchecked, would cause tremendous losses but because they can be detected, identified, and controlled on time with effective insecticides cause losses of about 10% of the crop; and weeds, which still are poorly controlled in much of the world because of unavailability of herbicides due to cost, cause losses of about 12% of the crop. The total crop loss from diseases and pests is estimated at about 36% or one-third of the potential production of the world. To these losses should be added 6–12% postharvest losses to pests, which brings the total (preharvest

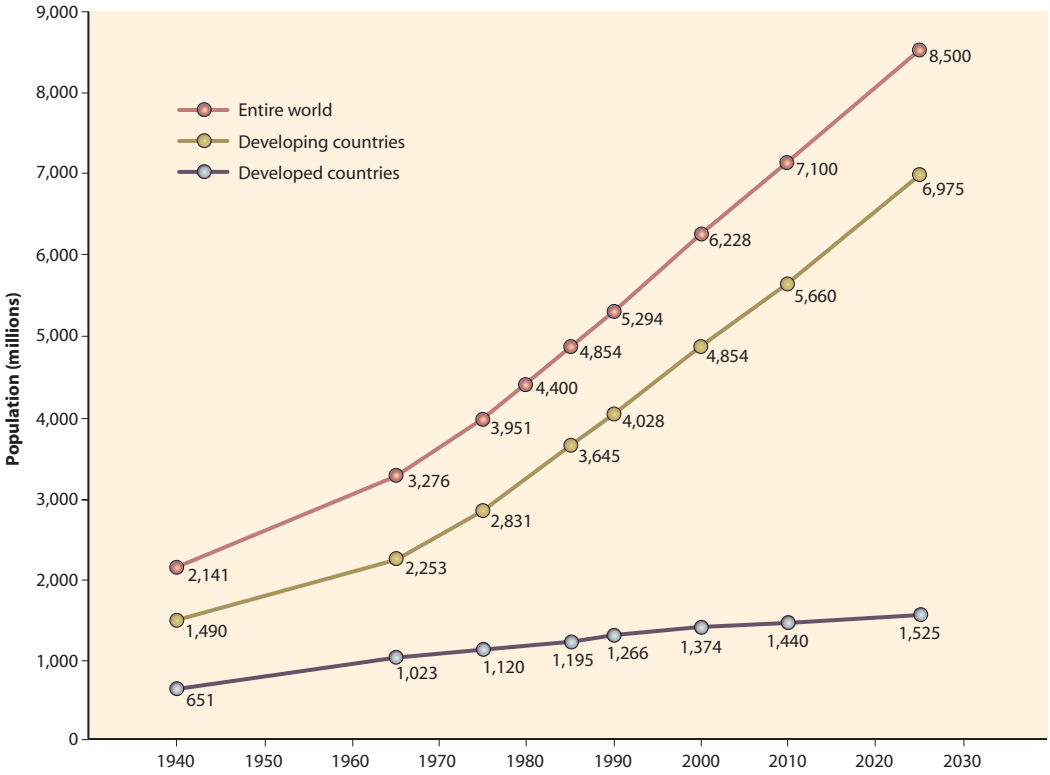

FIGURE 1-42 Real and projected population changes from 1940 to 2000 and to the year 2025. The rates of population growth were estimated for the years 1975 to 2000 and, for this graph, were assumed unchanged to the year 2025.

and postharvest) food losses to pests in the United States to about 40% and for the entire world to about 45% of all food crops. These losses occur, of course, despite all types of pest controls used. This is indeed a huge loss of needed food. It is apparent that losses are much greater in developing areas than they are in more developed ones. Another point that can be made is that insects cause much greater losses than diseases in developing countries, especially in Asia, because insects are controlled much more easily in developed countries than in developing ones, whereas losses caused by diseases seem to be as great in developed countries as they are in developing countries.

Crop losses caused by diseases, insects, and weeds become particularly striking and alarming when one considers their distribution among countries of varying degrees of development. In developed areas (Europe, North America, Australia, New Zealand, Japan, Israel, and South Africa), in which only 8.8% of the population is engaged in agriculture, the estimated losses and percentages of losses are considerably lower than those in developing countries, i.e., the rest of the world, in which 56.8% of the population is engaged in agriculture. The situation becomes particularly painful if one considers the fact that developing countries, which have much greater populations than developed countries, produce relatively less food and fiber and suffer much greater losses to plant diseases and to other pests. Taking into account the kinds of crops grown in temperate climates, where most developed countries are, and in the tropics, where developing countries are located, the total percentage losses differ considerably with the continent, as shown in Table 1-4. What is disheartening is that the more recent estimates by Oerke *et al.* (1994) indicate that the proportion of crop produce lost to diseases, insects, and weeds has actually increased in all continents (Table 1-4), despite presumably better and more widely used control materials and methods.

It is estimated that the total annual production for all agricultural crops worldwide is about $1500 billion (U.S. dollars, 2002). Of this, about $550 billion worth of produce is lost annually to diseases, insects, and weeds. An additional loss of about $455 billion would occur annually, but is averted by the use of various crop protection practices. Approximately $38 billion is spent annually for pesticides alone (fungicides, insecticides, herbicides), primarily in western Europe and in North America.

TABLE 1-4
Percentage of All Produce (1967 Estimate) and of Eight Major Crops (1994 Estimate) Lost to Diseases, Insects, and Weeds by Continent or Region[a]

	Produce lost to diseases, insects, and weeds (%)	
Continent or region	1967 estimate[b]	1994 Estimate[c]
Europe	25	28.2
Oceania	28	36.2
North and Central America	29	31.2
Russia and China	30	40.9
South America	33	41.3
Africa	42	48.9
Asia	43	47.1

[a]Reprinted from Oerke *et al.* (1994). The crops included are rice, wheat, barley, maize, potatoes, soybeans, cotton, and coffee.
[b]From H. H. Cramer (1967).
[c]The average worldwide loss to diseases, insects, and weeds was estimated at 42.1%.

Pesticides and Plant Diseases

The weed killers used increasingly in cultivated fields may cause injury to cultivated crop plants directly, but they also influence several soil pathogens and soil microorganisms antagonistic to pathogens. Other chemicals, such as fertilizers, insecticides, and fungicides, alter the types of microorganisms that survive and thrive in the soil, which sometimes leads to a reduction in the number of useful predators and antagonistic microorganisms of pathogens or their vectors. The use of fungicides and other pesticides specific against a particular pathogen often leads to increased populations and disease severity caused by other pathogens not affected by the specific pesticide. This occurs even with some rather broad-spectrum systemic fungicides that control most but not all pathogens, e.g., benomyl. Where such fungicides are used regularly and widely, some fungi, such as *Pythium*, that are not affected by them, may become more important as pathogens than when other more general fungicides are used.

The use of pesticides to control plant diseases and other pests had been, for many years since the mid–1950s, increasing steadily at an annual rate of about 14% (Fig. 1-43A). By 1999, nearly 2.6 billion kilograms (5.7 billion lbs) of active ingredients of pesticides were used per year worldwide at an annual cost of nearly $36 billion (Figs. 1-43B and 1-43C). In the United States alone, more than 550 million kg (1244 million lbs) of pesticides worth $11.2 billion (Figs. 1-43B–1-43E) were used in 1999. The relative amounts of active ingredient of herbicides, insecticides, fungicides, and other pesticides used in the United States and the world in 1998 or 1999 are shown in Figs. 1-43B–1-43E. Up to 1995, about 35% of all pesticides were applied in the United States and Canada, 45% in Europe, and the remaining 20% in the rest of the world. In the last several years, the use of pesticides has begun to decline in the United States and Europe, but as more countries become developed and can afford to buy pesticides, the use of pesticides in developing countries continues to increase sharply.

A large industry of pesticide research, production, and marketing has developed in the United States and some of the other countries. There are also hundreds of thousands of people who apply pesticides on crops as needed. The amount of pesticides applied on crops and the number of pesticide applicators varies considerably from region to region. This depends on the size of agriculture in the region, the climate of the region, and the kinds of crops grown in each. The Environmental Protection Agency has grouped the United States into 10 agricultural regions (Fig. 1-44A) and has estimated that the number of private pesticide applicators (i.e., individual farmers) and of commercial pesticide applicators varies from about 10,000 in some regions (No.1, New England states) to more than 300,000 in other regions (No.4, southeastern United States) (Fig. 1-44B).

There is little doubt that pesticide use has increased the yields of crops in most cases in which they have been applied. The cost of production, distribution, and application of pesticides is, of course, another form of economic loss caused by plant diseases and pests (Table 1-4). Furthermore, such huge amounts of poisonous substances damage the environment and food as they are spread over the crop plants several times each year. There are also the issues of worker protection from exposure to pesticides and poisonings of workers and consumers from pesticides.

Public awareness of the direct, indirect, and cumulative effects of pesticides on organisms other than the pests they are intended to control has led to increased emphasis on the protection of the environment. As a result, many pesticides have been abandoned or their use has been restricted, and their functions have been taken over by other less effective or more specific pesticides or by more costly or less efficient methods of control. The effort to control diseases and other pests by biological and cultural methods is still growing while at the same time more restrictions are being imposed on the testing, licensing, and application of pesticides. The pesticide producers must provide more detailed data on the effectiveness, toxicity, and persistence of each pesticide, and the application of each pesticide must be licensed for each crop on which it is going to be applied. Further-

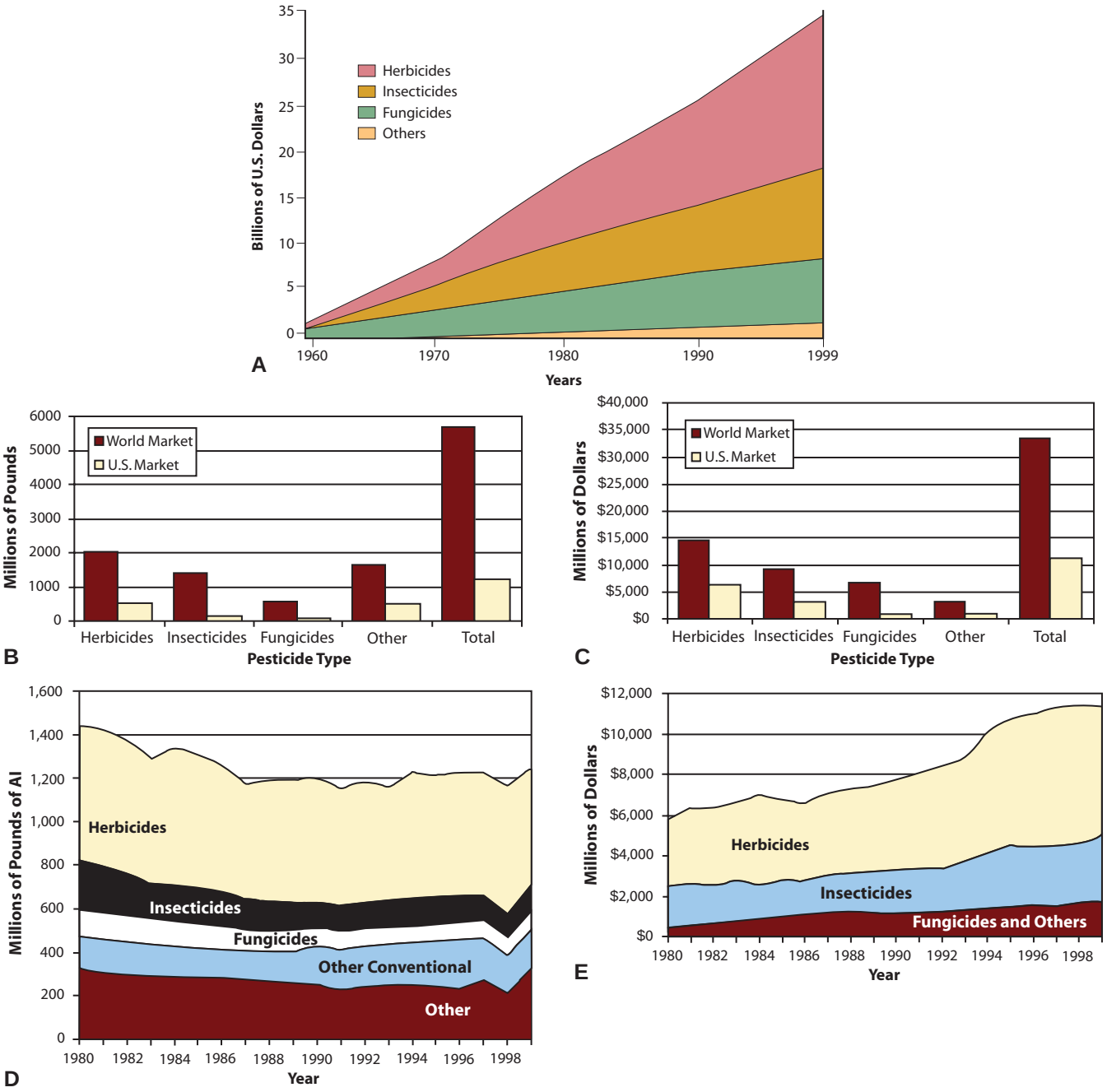

FIGURE 1-43 (A) Estimated worldwide annual sales of pesticides through 1999 in billions of dollars. Comparison of amounts of pesticides (in millions of pounds of active ingredient) used annually in the world and the United States (B) and of cost of pesticides (in millions of dollars) worldwide and the United States (C) at user level and by type of pesticide (B and C, 1999 estimates). (D) Annual usage in the United States of the various types of pesticides (in millions of pounds of active ingredient) from 1980 through 1999. (E) Cost of pesticides (in millions of dollars) spent annually in the United States from 1980 through 1999. Source: U.S. Environmental Protection Agency.

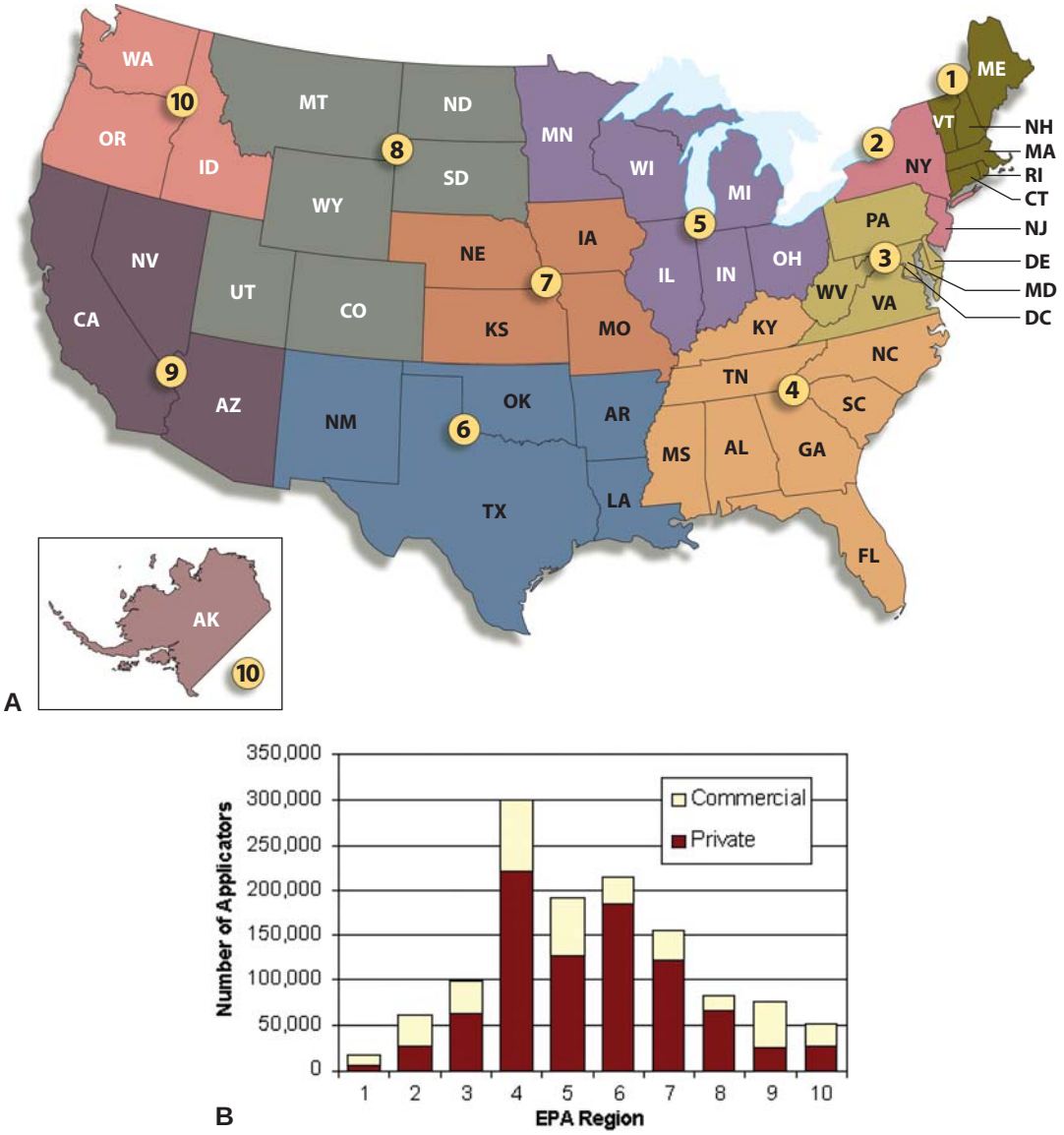

FIGURE 1-44 (A) Groups of states according to size and type of agriculture, and climate. (B) Numbers of private and commercial pesticide applicators in each region. Source: U.S. Environmental Protection Agency.

more, in some countries, each prospective commercial applicator of pesticides must pass an examination and be licensed to apply pesticides on crop plants. In some states, growers must clear with and get permission from state pest control advisors for the purchase and use of certain pesticides (prescription agriculture).

The desirability of using fewer and safer pesticides, however, is counteracted by the increasing demand of consumers over the last several decades for high-quality produce, especially fruits and vegetables free of any kind of blemishes caused by diseases or insects. A change in the attitude of consumers to demand less extravagant aesthetic quality of produce could reduce considerably the use of pesticides and the waste of perfectly wholesome foodstuffs, but such change in attitude may not occur for some time yet.

BASIC PROCEDURES IN THE DIAGNOSIS OF PLANT DISEASES

Pathogen or Environment

To diagnose a plant disease it is necessary to first determine whether the disease is caused by a pathogen or an environmental factor. In some cases, in which typical

symptoms of a disease or signs of the pathogen are present, it is fairly easy for an experienced person to determine not only whether the disease is caused by a pathogen or an environmental factor, but by which one. Frequently, comparing the symptoms with those given in books that list the known diseases and their causes for specific plant hosts or in books like those of the compendia series of the American Phytopathological Society helps narrow the number of likely causes and often helps identify the cause of the disease. In most cases, however, a detailed examination of the symptoms and an inquiry into characteristics beyond the obvious symptoms are necessary for a correct diagnosis.

Infectious Diseases

In diseases caused by pathogens (fungi, bacteria, parasitic higher plants, nematodes, viruses, mollicutes, and protozoa), a few or large numbers of these pathogens may be present on the surface of the plants (some fungi, bacteria, parasitic higher plants, and nematodes) or inside the plants (most pathogens). The presence of such pathogens on or in a plant indicates that they are probably the cause of the disease. Someone with experience can detect and identify pathogens, in some cases with the naked eye or with a magnifying lens (some fungi, all parasitic higher plants, some nematodes). More frequently, identification can be accomplished only by microscopic examination (fungi, bacteria, and nematodes) (see Fig. 1-3). If no such pathogens are present on the surface of a diseased plant, then one must look for additional symptoms and, especially, for pathogens inside the diseased plant. Such pathogens are usually at the margins of the affected tissues, at the vascular tissues, at the base of the plant, and on or in its roots.

Diseases Caused by Parasitic Higher Plants

The presence of a parasitic higher plant (e.g., dodder, mistletoe, witchweed, or broomrape) growing on a plant is sufficient for diagnosis of the disease.

Diseases Caused by Nematodes

If a plant parasitic nematode is present on, in, or in the rhizosphere of a plant showing certain kinds of symptoms, the nematode may be the pathogen that caused the disease or at least was involved in the production of the disease. If the nematode can be identified as belonging to a species or genus known to cause such a disease, then the diagnosis of the disease can be made with a degree of certainty.

Diseases Caused by Fungi and Bacteria

When fungal mycelia and spores, or bacteria, are present on the affected area of a diseased plant, two possibilities must be considered: (1) the fungus or bacterium may be the actual cause of the disease or (2) the fungus or bacterium may be one of the many saprophytic fungi or bacteria that can grow on dead plant tissue once the latter has been killed by some other cause, perhaps by even other fungi or bacteria.

Fungi

To determine whether a fungus found on or in a diseased plant is a pathogen or a saprophyte, one first studies under a microscope the morphology of its mycelium, fruiting structures, and spores. The fungus can then be identified and checked in an appropriate book of mycology or plant pathology to see whether it has been reported to be pathogenic, especially on the plant on which it was found. If the symptoms of the plant correspond to those listed in the book as caused by that particular fungus, then the diagnosis of the disease is, in most cases, considered complete. If no such fungus is known to cause a disease on plants, especially one with symptoms similar to the ones under study, then the fungus found should be considered a saprophyte or, possibly, a previously unreported plant pathogen, and the search for the proof of the cause of the disease must continue. In many cases, neither fruiting structures nor spores are initially present on diseased plant tissue, and therefore no identification of the fungus is possible. For some fungi, special nutrient media are available for selective isolation, identification, or promotion of sporulation. Others need to be incubated under certain temperature, aeration, or light conditions to produce spores. With most fungi, however, fruiting structures and spores are produced in the diseased tissue if the tissue is placed in a glass or plastic "moisture chamber," i.e., a container to which wet paper towels are added to increase the humidity in the air of the container.

Bacteria and Mollicutes

Diagnosis of a bacterial disease and identification of the causal bacterium is based primarily on the symptoms of the disease, the constant presence of large numbers of bacteria in the affected area, and the absence of any other pathogens. Bacteria are small (0.8 by 1 mm), however, and although they can be seen with a compound microscope, they all resemble tiny rods and have no distinguishing morphological characteristics for identification. Care must be taken, therefore, to exclude

the possibility that the observed bacteria are secondary saprophytes, i.e., bacteria that are growing in tissue killed by some other cause. Selective media are available for the selective cultivation of almost all plant pathogenic bacteria free of common saprophytes so that the genus and even some species can be identified. The easiest and surest way to prove that the observed bacterium is the pathogen is through isolation and growth of the bacterium in pure culture and, using a single colony for reinoculation of a susceptible host plant, reproducing the symptoms of the disease and comparing them with those produced by known species of bacteria. Since the late 1970s, immunodiagnostic techniques, including agglutination and precipitation, fluorescent antibody staining, and enzyme-linked immunosorbent assay, have been used to detect and identify plant pathogenic bacteria. Such techniques are quite sensitive, fairly specific, rapid, and easy to perform, and it is expected that soon standardized, reliable antisera will be available for serodiagnostic assays of plant pathogenic bacteria.

Since 1980, newer techniques have been used involving an automated analysis of fatty acid profiles of the bacteria or of the substances utilized by the bacteria for food (Biolog). Additional identification tests include comparison of the number of DNA pieces released by certain restriction enzymes, or degrees (percentages) of hybridization of the DNA of an unknown bacterium with the DNA of a known one. Some of the molecular techniques are now used for the identification of fastidious vascular bacteria.

Diseases caused by mollicutes appear as stunting of plants, yellowing or reddening of leaves, proliferation of shoots and roots, production of abnormal flowers, and eventual decline and death of the plant. Mollicutes are small, polymorphic, wall-less bacteria that live in young phloem cells of their hosts; they are generally visible only under an electron microscope and, except for the genus *Spiroplasma*, cannot be cultured on nutrient media. The diagnosis of such diseases, therefore, is based on symptomatology, graft transmissibility, transmission by certain insect vectors, electron microscopy, sensitivity to tetracycline antibiotics but not to penicillin, sensitivity to moderately high (32–35.8°C) temperatures, and, in a few cases in which specific antisera have been prepared, on serodiagnostic tests.

Diseases Caused by Viruses and Viroids

Many viruses (and viroids) cause distinctive symptoms in their hosts, and so the disease and the virus (or viroid) can be identified quickly by the symptoms. In the many other cases in which this is not possible, however, the diseases are diagnosed and the viruses are identified primarily as follows: (1) through virus transmission tests to specific host plants by sap inoculation or by grafting, and sometimes by certain insect, nematode, fungus, or mite vectors; (2) for viruses for which specific antisera are available, by using serodiagnostic tests, primarily enzyme-linked immunosorbent assays (ELISA), gel diffusion tests, microprecipitin tests, and fluorescent antibody staining; (3) by electron microscopy techniques such as negative staining of virus particles in leaf dip or purified preparations, or immune-specific electron microscopy (a combination of serodiagnosis and electron microscopy); (4) by microscopic examination of infected cells for specific crystalline or amorphous inclusions, which usually are diagnostic of the group to which the virus belongs; (5) through electrophoretic tests, useful primarily for detection and diagnosis of viroids and of nucleic acids of viruses; and (6) via hybridization of commercially available radioactive DNA complementary to a certain virus DNA or RNA, or viroid RNA, with the DNA or RNA present in plant sap and attached to a membrane filter (immunoblot).

Diseases Caused by More Than One Pathogen

Quite frequently a plant may be attacked by two or more pathogens of the same or different kinds and may develop one or more types of disease symptoms. It is important to recognize the presence of the additional pathogen(s). Once this is ascertained, the diagnosis of the disease(s) and the identification of the pathogen(s) proceed as described earlier for each kind of pathogen.

Noninfectious Diseases

If no pathogen can be found, cultured, or transmitted from a diseased plant, then it must be assumed that the disease is caused by an abiotic environmental factor. The number of environmental factors that can cause disease in plants is almost unlimited, but most of them affect plants by interfering with normal physiological processes. Such interference may be a result of an excess of a toxic substance in the soil or in the air, a lack of an essential substance (water, oxygen, or mineral nutrients), or a result of an extreme in the conditions supporting plant life (temperature, humidity, oxygen, CO_2, or light). Some of these effects may be the result of normal conditions (e.g., low temperatures) occurring at the wrong time or of abnormal conditions brought about naturally (flooding or drought) or by the activi-

ties of people and their machines (air pollutants, soil compaction, and weed killers).

The specific environmental factor that has caused a disease might be determined by observing a change in the environment, e.g., a flood or an unseasonable frost. Some environmental factors cause specific symptoms on plants that help determine the cause of the malady, but most of them cause nonspecific symptoms that, unless the history of the environmental conditions is known, make it difficult to diagnose the cause accurately.

Identification of a Previously Unknown Disease: Koch's Rules (Postulates)

When a pathogen is found on a diseased plant, the pathogen is identified by reference to special manuals; if the pathogen is known to cause such a disease and the diagnostician is confident that no other causal agents are involved, then the diagnosis of the disease may be considered completed. If, however, the pathogen found seems to be the cause of the disease but no previous reports exist to support this, then the steps described on page 27 under Koch's postulates are taken to verify the hypothesis that the isolated pathogen is the cause of the disease

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chapter two

PARASITISM *AND* DISEASE DEVELOPMENT

The pathogens that attack plants belong to the same groups of organisms that cause diseases in humans and animals. Moreover, plants are attacked by a number of other plants. With the exception of some insect-transmitted plant pathogens, however, which cause diseases in both their host plants and their insect vectors, none of the pathogen species that attack plants is known to affect humans or animals.

Infectious diseases are those that result from infection of a plant by a pathogen. In such diseases, the pathogen can grow and multiply rapidly on diseased plants, it can spread from diseased to healthy plants, and it can cause additional plants to become diseased, thereby leading to the development of a small or large epidemic.

PARASITISM AND PATHOGENICITY

An organism that lives on or in some other organism and obtains its food from the latter is called a **parasite.** The removal of food by a parasite from its host is called **parasitism**. A **plant parasite** is an organism that becomes intimately associated with a plant and multiplies or grows at the expense of the plant. The removal by the parasite of nutrients and water from the host plant usually reduces efficiency in the normal growth of the plant and becomes detrimental to the further development and reproduction of the plant. In many cases, parasitism is intimately associated with **pathogenicity**, i.e., the ability of a pathogen to cause disease, as the ability

of the parasite to invade and become established in the host generally results in the development of a diseased condition in the host.

In some cases of parasitism, as with the root nodule bacteria of legume plants and the mycorrhizal infection of feeder roots of most flowering plants, both the plant and the microorganism benefit from the association. This phenomenon is known as **symbiosis**.

In most plant diseases, however, the amount of damage caused to plants is often much greater than would be expected from the mere removal of nutrients by the parasite. This additional damage results from substances secreted by the parasite or produced by the host in response to stimuli originating in the parasite. Tissues affected by such substances may show increased respiration, disintegration or collapse of cells, wilting, abscission, abnormal cell division and enlargement, and degeneration of specific components such as chlorophyll. These conditions in themselves do not seem directly to improve the welfare of the parasite. It would appear, therefore, that the damage caused by a parasite is not always proportional to the nutrients removed by the parasite from its host. **Pathogenicity**, then, is the ability of the parasite to interfere with one or more of the essential functions of the plant, thereby causing disease. Parasitism frequently plays an important, but not always the most important, role in pathogenicity.

Of the large number of groups of living organisms, only a few members of a few groups can parasitize plants: fungi, bacteria, mollicutes, parasitic higher plants, parasitic green algae, nematodes, protozoa, viruses, and viroids. These parasites are successful because they can invade a host plant, feed and proliferate in it, and withstand the conditions in which the host lives. Some parasites, including viruses, viroids, mollicutes, some fastidious bacteria, nematodes, protozoa, and fungi causing downy mildews, powdery mildews, and rusts, are **biotrophs**, i.e., they can grow and reproduce in nature only in living hosts, and they are called **obligate parasites**. Other parasites (most fungi and bacteria) can live on either living or dead hosts and on various nutrient media, and they are therefore called **nonobligate parasites**. Some nonobligate parasites live most of the time or most of their life cycles as parasites, but, under certain conditions, may grow saprophytically on dead organic matter; such parasites are **semibiotrophs** and are called **facultative saprophytes**. Others live most of the time and thrive well on dead organic matter (**necrotrophs**) but, under certain circumstances, may attack living plants and become parasitic; these parasites are called **facultative parasites**. Usually no correlation exists between the degree of parasitism of a pathogen and the severity of disease it can cause, as many diseases caused by weakly parasitic pathogens are much more damaging to a plant than others caused even by obligate parasites. Moreover, certain pathogens, e.g., slime molds and those causing sooty molds, can cause disease by just covering the surface of the plant without parasitizing the plant.

Obligate and nonobligate parasites generally differ in the ways in which they attack their host plants and procure their nutrients from the host. Many nonobligate parasites secrete enzymes that bring about the disintegration of the cell components of plants, and these alone or with the toxins secreted by the pathogen result in the death and degradation of the cells. The invading pathogen then utilizes the contents of the cells for its growth. Many fungi and most bacteria act in this fashion, growing as necrotrophs on a nonliving substrate within a living plant. This mode of nutrition is like that of saprophytes. However, all obligate (and some nonobligate) parasites do not kill cells in advance but get their nutrients either by penetrating living cells or by establishing close contact with them. The association of these pathogens with their host cells is an intimate one and results in continuous absorption or diversion of nutrients, which would normally be utilized by the host, into the body of the parasite. The depletion of nutrients, however, although it restricts the growth of the host and causes symptoms, does not always kill the host. In the case of obligate parasites, death of the host cells restricts the further development of the parasite and may result in its death.

Parasitism of cultivated crops is a common phenomenon. In North America, for example, more than 8,000 species of fungi cause nearly 100,000 diseases, and at least 200 bacteria, about 75 mollicutes, more than 1,000 different viruses and 40 viroids, and more than 500 species of nematodes attack crops. Although about 2,500 species of higher plants are parasitic on other plants, only a few of them are serious parasites of crop plants. A single crop, e.g., the tomato, may be attacked by more than 40 species of fungi, 7 bacteria, 16 viruses, several mollicutes, and several nematodes. This number of diseases is average as corn has 100, wheat 80, and apple and potato each are susceptible to about 80–100 diseases. Fortunately, however, in any given location, only a fraction of the diseases affecting a crop are present and, in any given year, only a small number of these diseases become severe.

HOST RANGE OF PATHOGENS

Pathogens differ with respect to the kinds of plants that they can attack, with respect to the organs and tissues that they can infect, and with respect to the age of the organ or tissue of the plant on which they can grow.

Some pathogens are restricted to a single species, others to one genus of plants, and still others have a wide range of hosts, belonging to many families of higher plants. Some pathogens grow especially on roots, others on stems, and some mainly on the leaves or on fleshy fruits or vegetables. Some pathogens, e.g., vascular parasites, attack specifically certain kinds of tissues, such as phloem or xylem. Others may produce different effects on different parts of the same plant. With regard to the age of plants, some pathogens attack seedlings or the young tender parts of plants, whereas others attack only mature tissues.

Many obligate parasites are quite specific as to the kind of host they attack, possibly because they have evolved in parallel with their host and require certain nutrients that are produced or become available to the pathogen only in these hosts. However, many viruses and nematodes, although obligate parasites, attack many different host plants. Nonobligate parasites, especially root, stem, and fruit-attacking fungi, usually attack many different plants and plant parts of varying age, possibly because these pathogens depend on nonspecific toxins or enzymes that affect substances or processes found commonly among plants for their attack. Some nonobligate parasites, however, produce disease on only one or a few plant species. In any case, the number of plant species currently known to be susceptible to a single pathogen is smaller than the actual number in nature, as only a few species out of thousands have been studied for their susceptibility to each pathogen. Furthermore, because of genetic changes, a pathogen may be able to attack hosts previously immune to it. It should be noted, however, that each plant species is susceptible to attack by only a relatively small number of all known plant pathogens.

DEVELOPMENT OF DISEASE IN PLANTS

A plant becomes diseased in most cases when it is attacked by a pathogen or when it is affected by an abiotic agent. Therefore, in the first case, for a plant disease to occur, at least two components (plant and pathogen) must come in contact and must interact. If at the time of contact of a pathogen with a plant, and for some time afterward, conditions are too cold, too hot, too dry, or some other extreme, the pathogen may be unable to attack or the plant may be able to resist the attack, and therefore, despite the two being in contact, no disease develops. Apparently then, a third component, namely a set of environmental conditions within a favorable range, must also occur for disease to develop. Each of the three components can display considerable variability; however, as one component changes it affects the degree of disease severity within an individual plant and within a plant population. For example, the plant may be of a species or variety that may be more or less resistant to the pathogen or it may be too young or too old for what the pathogen prefers, or plants over a large area may show genetic uniformity, all of which can either reduce or increase the rate of disease development by a particular pathogen. Similarly, the pathogen may be of a more or less virulent race, it may be present in small or extremely large numbers, it may be in a dormant state, or it may require a film of water or a specific vector. Finally, the environment may affect both the growth and the resistance of the host plant and also the rate of growth or multiplication and degree of virulence of the pathogen, as well as its dispersal by wind, water, vector, and so on.

The interactions of the three components of disease have often been visualized as a triangle (Fig. 2-1), generally referred to as the "**disease triangle**." Each side of the triangle represents one of the three components. The length of each side is proportional to the sum total of the characteristics of each component that favor disease. For example, if the plants are resistant, the wrong age, or widely spaced, the host side — and the amount of disease — would be small or zero, whereas if the plants are susceptible, at a susceptible stage of growth, or planted densely, the host side would be long and the potential amount of disease could be great. Similarly, the more virulent, abundant, and active the pathogen, the longer the pathogen side would be and the greater the potential amount of disease. Also, the more favorable the environmental conditions that help the pathogen (e.g., temperature, moisture, and wind) or that reduce host resistance, the longer the environment side would be and the greater the potential amount of disease. If the three components of the disease triangle could be quantified, the area of the triangle would represent the amount of disease in a plant or in a plant population. If any of the three components is zero, there can

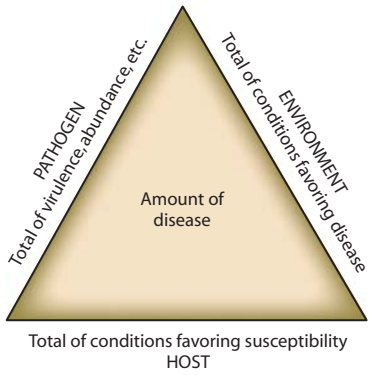


FIGURE 2-1 The disease triangle.

be no disease. The disease triangle is also represented as a triangle with the words of the three components (host plant, pathogen, environment) placed at the peaks of the triangle rather than along its sides.

STAGES IN THE DEVELOPMENT OF DISEASE: THE DISEASE CYCLE

In every infectious disease a series of more or less distinct events occurs in succession and leads to the development and perpetuation of the disease and the pathogen. This chain of events is called a **disease cycle**. A disease cycle sometimes corresponds fairly closely to the **life cycle** of the pathogen, but it refers primarily to the appearance, development, and perpetuation of the disease as a function of the pathogen rather than to the pathogen itself. The disease cycle involves changes in the plant and its symptoms as well as those in the pathogen and spans periods within a growing season and from one growing season to the next. The primary events in a disease cycle are inoculation, penetration, establishment of infection, colonization (invasion), growth and reproduction of the pathogen, dissemination of the pathogen, and survival of the pathogen in the absence of the host, i.e., overwintering or oversummering (overseasoning) of the pathogen (Fig. 2-2). In some diseases there may be several **infection cycles** within one disease cycle.

Inoculation

Inoculation is the initial contact of a pathogen with a site of plant where infection is possible. The pathogen(s) that lands on or is otherwise brought into contact with the plant is called the **inoculum**. The inoculum is any part of the pathogen that can initiate infection. Thus, in fungi the inoculum may be spores (Figs. 2-3A–2-3C), **sclerotia** (i.e., a compact mass of mycelium), or fragments of mycelium. In bacteria, mollicutes, protozoa, viruses, and viroids, the inoculum is always whole individuals of bacteria (Fig. 2-3D), mollicutes, protozoa, viruses, and viroids, respectively. In nematodes, the inoculum may be adult nematodes, nematode juveniles, or eggs. In parasitic higher plants, the inoculum may be plant fragments or seeds. The inoculum may consist of a single individual of a pathogen, e.g., one spore or one multicellular sclerotium, or of millions of individuals of a pathogen, e.g., bacteria carried in a drop of water. One unit of inoculum of any pathogen is called a **propagule**.

Types of Inoculum

An inoculum that survives dormant in the winter or summer and causes the original infections in the spring or in the autumn is called a **primary inoculum**, and the infections it causes are called **primary infections**. An inoculum produced from primary infections is called a **secondary inoculum** and it, in turn, causes **secondary infections**. Generally, the more abundant the primary inoculum and the closer it is to the crop, the more severe the disease and the losses that result.

Sources of Inoculum

In some fungal and bacterial diseases of perennial plants, such as shrubs and trees, the inoculum is produced on the branches, trunks, or roots of the plants. The inoculum sometimes is present right in the plant

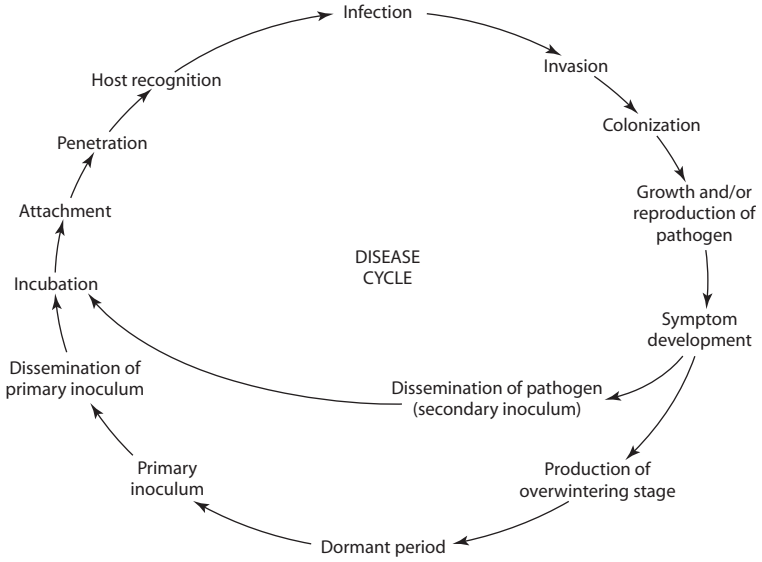


FIGURE 2-2 Stages in development of a disease cycle.

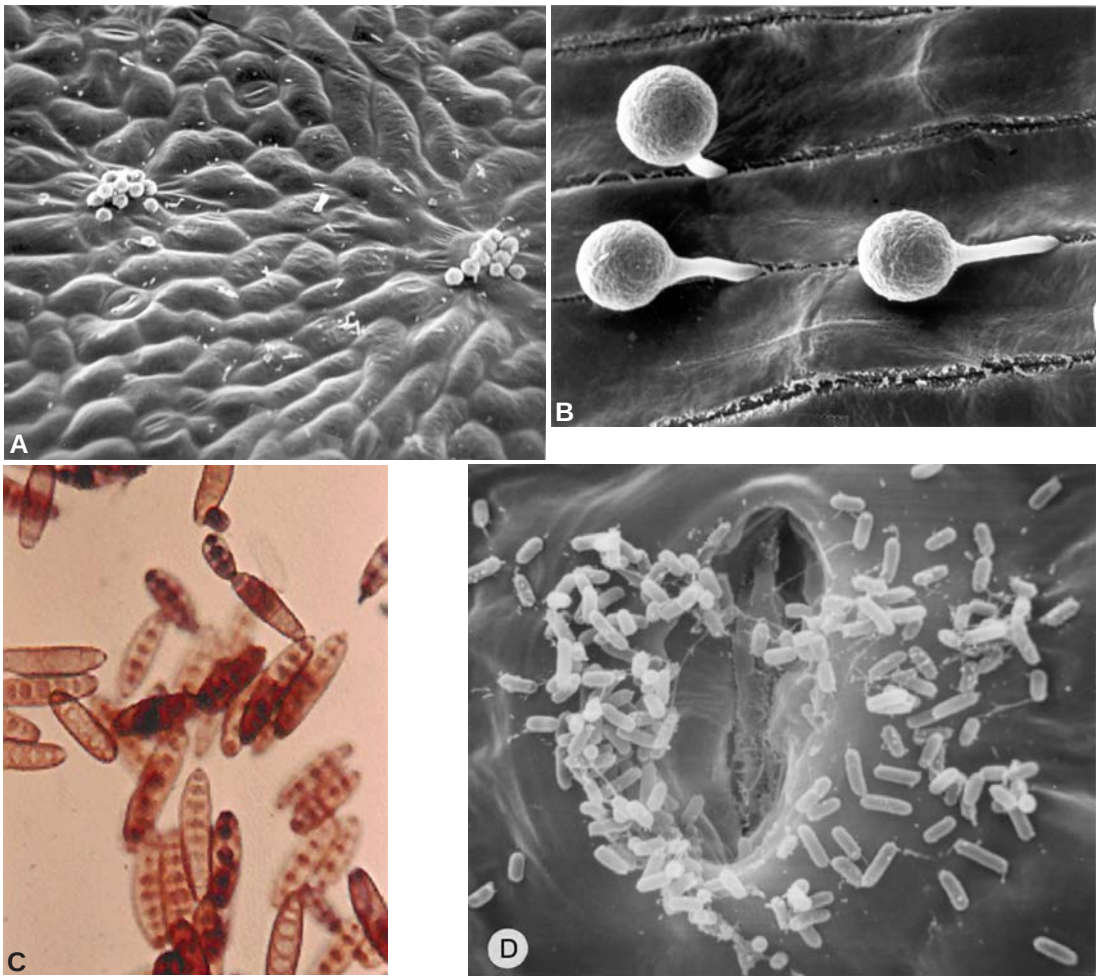


FIGURE 2-3 Types of inoculum and ways in which some pathogens enter a host plant. (A) Two groups of zoospores of the grape downy mildew oomycete have gathered over two leaf stomata. (B) Encysted zoospores of the soybean root rot pathogen *Phytophthora sojae* germinating and penetrating the root. (C) Mitospores (conidia) of a fungus that causes a corn leaf spot disease. (D) Bacteria of *Pseudomonas syringae* that causes bacterial spot and canker of stone fruits are seen in and surrounding a stoma of a cherry leaf. [Photographs courtesy of (A) D. J. Royle, (B) C. W. Mims and K. Enkerli, University of Georgia, and (D) E. L. Mansvelt, Stellenbosch, South Africa.]

debris or soil in the field where the crop is grown; other times it comes into the field with the seed, transplants, tubers, or other propagative organs or it may come from sources outside the field. Outside sources of inoculum may be nearby plants or fields or fields many miles away. In many plant diseases, especially those of annual crops, the inoculum survives in perennial weeds or alternate hosts, and every season it is carried from them to the annual and other plants. Fungi, bacteria, parasitic higher plants, and nematodes either produce their inoculum on the surface of infected plants or their inoculum reaches the plant surface when the infected tissue breaks down. Viruses, viroids, mollicutes, fastidious bacteria, and protozoa produce their inoculum within the plants; such an inoculum almost never reaches the plant surface in nature and, therefore, it can be transmitted from one plant to another almost entirely by some kind of vector, such as an insect.

Landing or Arrival of Inoculum

The inoculum of most pathogens is carried to host plants passively by wind, water, and insects. An airborne inoculum usually gets out of the air and onto the plant surface not just by gravity but by being washed out by rain. Only a tiny fraction of the potential inoculum produced actually lands on susceptible host plants; the bulk of the produced inoculum lands on things that cannot

become infected. Some types of inoculum in the soil, e.g., zoospores and nematodes, may be attracted to the host plant by such substances as sugars and amino acids diffusing out of the plant roots. Vector-transmitted pathogens are usually carried to their host plants with an extremely high efficiency.

Prepenetration Phenomena

Attachment of Pathogen to Host

Pathogens such as mollicutes, fastidious bacteria, protozoa, and most viruses are placed directly into cells of plants by their vectors and, in most cases, they are probably immediately surrounded by cytoplasm, cytoplasmic membranes, and cell walls. However, almost all fungi, bacteria, and parasitic higher plants are first brought into contact with the external surface of plant organs. Before they can penetrate and colonize the host, they must first become attached to the host surface (Figs. 2-3–2-6). Attachment takes place through the adhesion of spores, bacteria, and seeds through adhesive materials that vary significantly in composition and in the environmental factors they need to become adhesive. Disruption of adhesion by nontoxic synthetic compounds results in failure of the spores to infect leaves.

The propagules of these pathogens have on their surface or at their tips mucilaginous substances consisting of mixtures of water-insoluble polysaccharides, glycoproteins, lipids, and fibrillar materials, which, when moistened, become sticky and help the pathogen adhere to the plant. In some fungi, hydration of the spore by moist air or dew causes the extrusion of preformed mucilage at the tip of the spore that serves for the immediate adherence of the spore to the hydrophobic plant surface and resistance to removal by flowing water. However, in powdery mildew fungi, which do not require free water for infection, adhesion is accomplished by release from the spore of the enzyme cutinase, which makes the plant and spore areas of attachment more hydrophilic and cements the spore to the plant surface. In other cases, propagule adhesion requires on-the-spot synthesis of new glycoproteins and it may not reach maximum levels until 30 minutes after contact. In some fungi causing vascular wilts, spores fail to adhere after hydration but become adhesive after they are allowed to respire and to synthesize new proteins.

How exactly spores adhere to plant surfaces is not known, but it seems to either involve a very specific interaction of the spore with a host plant surface via lectins, ionic interactions, or hydrophobic contact with the plant cuticle, or involve stimulation of the spore by physical rather than chemical signals. The extracellular matrix surrounding the propagules of many pathogens contains several enzymes, including cutinases, which are expected to play an important role in spore attachment. In any case, the act of attachment often seems necessary for the subsequent transmission of signals for germ tube extension and production of infection structure. It is now clear that many proteins of the fungal cell wall, in addition to their structural role, play an important role in the adhesion of fungi, as well as in the host-surface perception by the fungus.

Spore Germination and Perception of the Host Surface

It is not clear what exactly triggers spore germination, but stimulation by the contact with the host surface, hydration and absorption of low molecular weight ionic material from the host surface, and availability of nutrients play a role. Spores also have mechanisms that prevent their germination until they sense such stimulations or when there are too many spores in their vicinity. Once the stimulation for germination has been received by the spore, the latter mobilizes its stored food reserves, such as lipids, polyoles, and carbohydrates, and directs them toward the rapid synthesis of cell membrane and cell wall toward the germ tube formation and extension (Figs. 2-4 and 2-5). The germ tube is a specialized structure distinct from the fungal mycelium, often growing for a very short distance before it differentiates into an appressorium. The germ tube is also the structure and site that perceives the host surface and, if it does not receive the appropriate external stimuli, the germ tube remains undifferentiated and, when the nutrients are exhausted, it stops growing. When appropriate physical and chemical signals, such as surface hardness, hydrophobicity, surface topography, and plant signals, are present, germ tube extension and differentiation take place.

The perception of signals from plant surfaces by pathogenic fungi (Fig. 2-6) seems to be the result of signaling pathways mediated by cyclic adenosine monophosphate (cAMP) and mitogen-activated protein kinase (MAPK), which have been implicated in regulating the development of infection-related phenomena in many different fungi. In response to a signal from the host plant, e.g., the presence of a hydrophobic plant surface, which transmits a cue for appressorium formation, the fungus perceives the extracellular signal and its transmission via the plasma membrane and, as a first step, it accumulates intracellular signaling molecules and induces a phosphorylation cascade. In some fungi, the receptor of the signal is a protein in the plasma membrane of the fungal spore. Transmission of the cAMP signal proceeds via the cAMP-dependent activity of

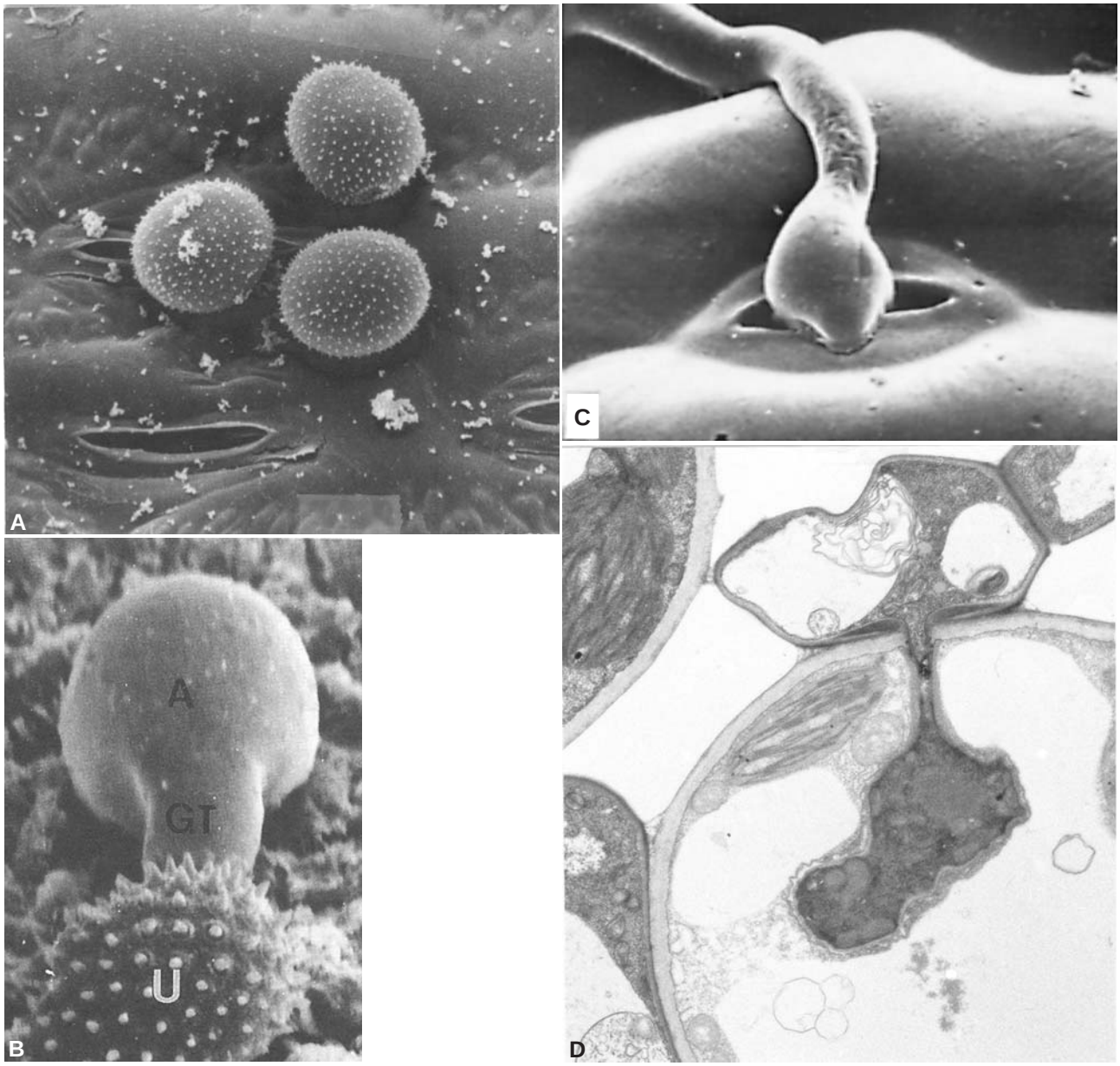


FIGURE 2-4 Methods of germination and penetration by fungi. (A) Uredospores of a rust fungus on a grass leaf next to open stomata. (B) A rust uredospore (U) that has germinated and produced a dome-like appressorium. (C) Uredospore germination, germ tube elongation, and appressorium penetration through a stoma. (D) A haustorium of a rust fungus inside a host cell. (E) A spore of the apple black rot fungus that has germinated directly into mycelium. (F) Two multicellular conidia of *Alternaria* sp. (G) A germinating conidium of *Alternaria* with a germ tube covered with extracellular material. [Photographs courtesy of (A) Plant Pathology Department, University of Florida, (B and C) W. K. Wynn and (D) C. W. Mims, University of Georgia, (E) J. Rytter and J. W. Travis, Pennslyvania State University, (F and G) Mims *et al.* (1997). *Can. J. Bot.* 75, 252–260.]

(continued on next page)

protein kinase A (= PKA) and subsequent phosphorylation of target proteins. The major activity of PKA in developing germ tubes is the mobilization of carbohydrates and lipids to the appressorium site and is, therefore, pivotal to the production of functional appressoria. In some fungi, cAMP signaling is required for the initiation of appressorium development, at which time intracellular cAMP concentrations rise during differentiation of conidia and emergence of the appressorium germ tube. Subsequently, cAMP levels fall as the germ tube extends and, if more cAMP is added at this point, further development of the germ tube is inhibited.

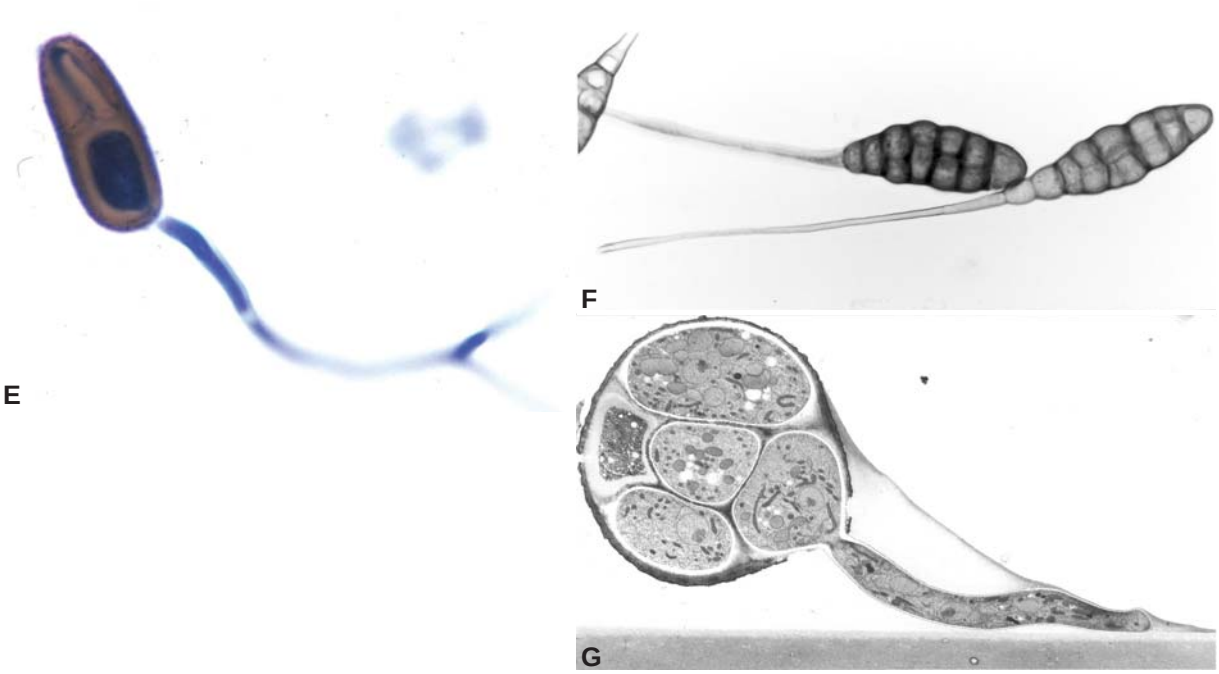


FIGURE 2-4 (*Continued*)

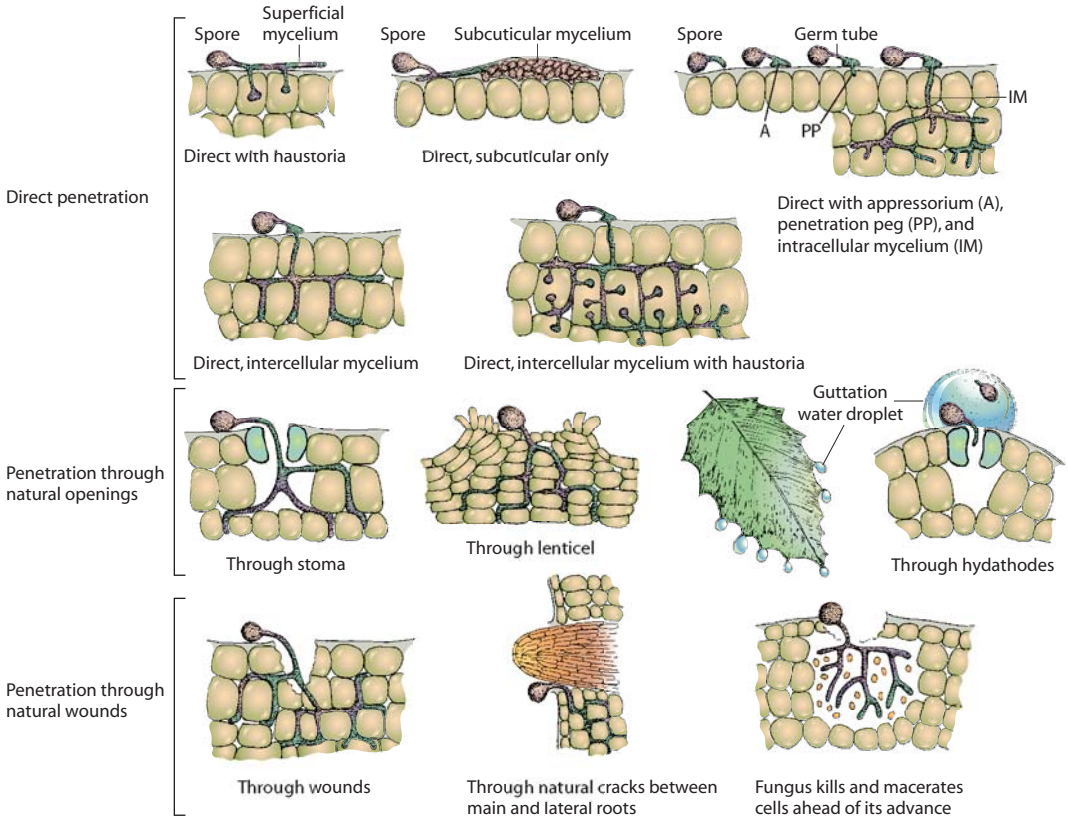


FIGURE 2-5 Methods of penetration and invasion by fungi.

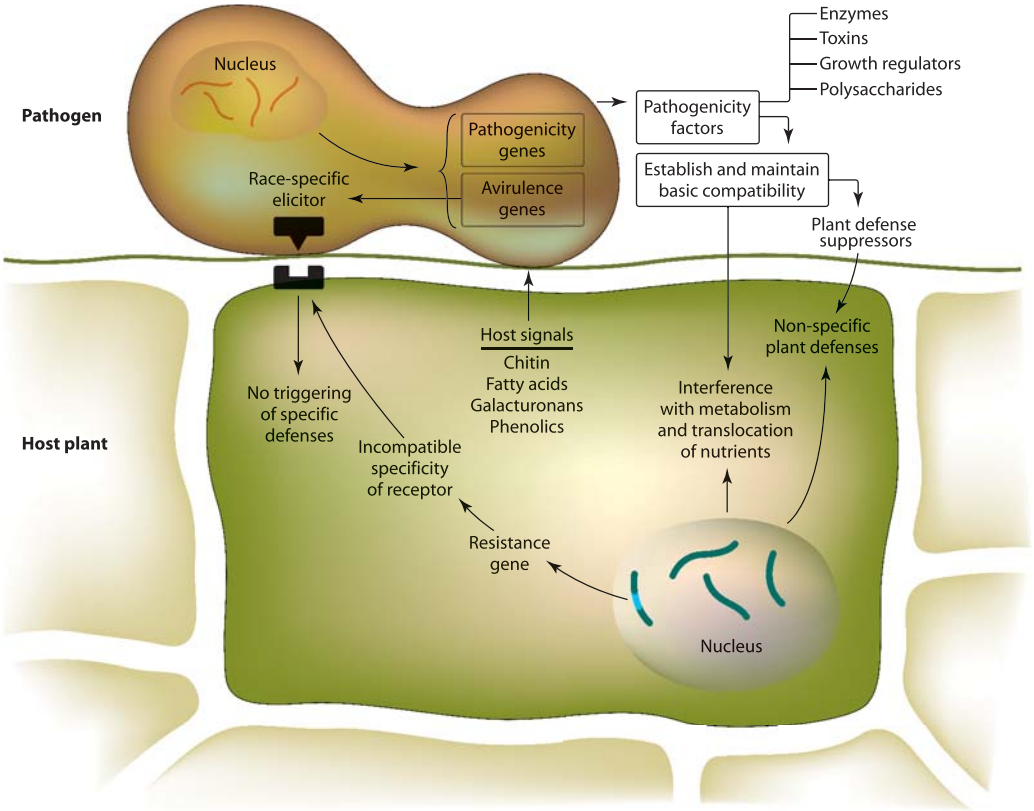


FIGURE 2-6 Establishment of infection in a compatible reaction between a pathogen and its host plant.

Signaling pathways for infection-related development are also achieved through mitogen-activated protein kinases (MAPKs) and their upstream regulatory kinases. All of these together comprise a functional unit that transmits input signals from the periphery of the cell to the cell nucleus to elicit the expression of appropriate genes. A MAP kinase, K1 or P1, regulates appressorium formation in response to a signal from the plant surface but it is also required for invasive growth or viability in its host plant.

After attachment of the propagule to the host surface, as spores and seeds germinate, germ tubes also produce mucilaginous materials that allow them to adhere to the cuticular surface of the host, either along their entire length or only at the tip of the germ tube. In regions of contact with the germ tube, the structure of the host cuticle and cell walls often appears altered, presumably as a result of degradative enzymes contained in the mucilaginous sheath.

Appressorium Formation and Maturation

Once appressoria are formed, they adhere tightly to the leaf surface (Figs. 2-4 and 2-9). Subsequently, appressoria secrete extracellular enzymes, generate physical force, or both to bring about penetration of the cuticle by the fungus. Appressoria must be attached to the host plant surface strongly enough to withstand the invasive physical force applied by the fungus and to resist the chemical action of the enzymes secreted by the fungus. Appressoria of some fungi contain lipids, polysaccharides, and proteins. Fungi that produce melanin-pigmented appressoria produce a narrow penetration hypha from the base of the appressorium and use primarily physical force to puncture the plant cuticle with that hypha.

The size of the turgor pressure inside an appressorium has been measured and found to be 40 times greater than the pressure of a typical car tire. The turgor pressure of an appressorium is due to the enormous accumulation of glycerol in the appressorium, which, due to its high osmotic pressure, draws water into the cell and generates hydrostatic pressure that pushes the thin hypha (appressorial penetration peg) outward through the host cuticle. Mobilization of spore-stored products to the developing appressorium and glycerol biosynthesis in it is regulated by the cAMP signaling pathway, whereas the initial movement of lipid and glycogen reserves to the developing appressorium was also found to be regulated by the K1 MAP. This

indicates that the maturation of appressoria and their specific biochemical activity are intimately associated with genetic control of the initial development of appressoria.

The production of penetration hyphae by appressoria, or directly from germ tubes, is not well understood at the genetic level. Production of the penetration peg requires the localization of actin to the hyphal tip and rapid biosynthesis of the cell wall as the hypha grows through the cuticle and the layers of the epidermal cell walls. Production of penetration hyphae appears to be regulated by a MAP kinase pathway.

Recognition between Host and Pathogen

It is still unclear how pathogens recognize their hosts and vice versa. It is assumed that when a pathogen comes in contact with a host cell, an early event takes place that triggers a fairly rapid response in each organism that either allows or impedes further growth of the pathogen and development of disease. The nature of the "early event" is not known with certainty in any host–parasite combination, but it may be one of many biochemical substances, structures, and pathways. These may include specific host signal compounds or structures, or specific pathogen elicitor molecules, and either of them may induce specific actions or formation of specific products by the other organism (Fig. 2-6).

Host components acting as signals for recognition by and activation of pathogens are numerous. They may include fatty acids of the plant cuticle that activate production by the pathogen of the cutinase enzyme, which breaks down cutin; galacturonan molecules of host pectin, which stimulate the production of pectin lyase enzymes by the fungus or bacterium; certain phenolic compounds, such as strigol, which stimulate activation and germination of propagules of some pathogens; and isoflavones and other phenolics, amino acids, and sugars released from plant wounds that activate a series of genes in certain pathogens leading to infection. A host plant may also send cues for recognition by some of its pathogens by certain of its surface characteristics such as ridges or furrows, hardness, or release of certain ions such as calcium.

Pathogen components that act as elicitors of recognition by the host plant and subsequent mobilization of plant defenses are still poorly understood. Elicitor molecules may be released from attacking pathogens before or during entry into the host, and they may have a narrow host range, e.g., the elicitins. Some elicitors may be components of the cell surface of the pathogen (e.g., β-glucans, chitin, or chitosan) that are released by the action of host enzymes (e.g., β-glucanase and/or chitinase) and have broad host ranges; some may be synthesized and released by the pathogen after it enters the host in response to host signals. The latter elicitors include the harpin proteins of bacteria that induce development of the hypersensitive response, certain hydroxylipids, and certain peptides and carbohydrates that induce specific host defense responses such as the production of phytoalexins. Elicitors are considered as determinants of pathogen avirulence, as by their presence they elicit the hypersensitive (resistance) response and initiation of transcription of the plant genes that encode the various components of the defense response. These defense measures by the host plant, in turn, result in the pathogen appearing as avirulent.

When the initial recognition signal received by the pathogen favors growth and development, disease may be induced; if the signal suppresses pathogen growth and activity, disease may be aborted. However, if the initial recognition elicitor received by the host triggers a defense reaction, pathogen growth and activity may be slowed or stopped and disease may not develop; if the elicitor either suppresses or bypasses the defense reaction of the host, disease may develop.

Germination of Spores and Seeds

Almost all pathogens in their vegetative state are capable of initiating infection immediately. Fungal spores and seeds of parasitic higher plants, however, must first germinate (Figs. 2-4 and 2-5). Spores germinate by producing a typical mycelium (Figs. 2-4E and 2-4G) that infects and grows into host plants or they produce a short germ tube that produces a specialized infectious structure, the haustorium (Figs. 2-4B–2-4D). In order to germinate, spores require a favorable temperature and also moisture in the form of rain, dew, or a film of water on the plant surface or at least high relative humidity. The moist conditions must last long enough for the pathogen to penetrate or else it desiccates and dies. Most spores can germinate immediately after their maturation and release, but others (so-called resting spores) require a dormancy period of varying duration before they can germinate. When a spore germinates it produces a germ tube, i.e., the first part of the mycelium, that can penetrate the host plant. Some fungal spores germinate by producing other spores, e.g., sporangia produce zoospores and teliospores produce basidiospores.

Spore germination is often favored by nutrients diffusing from the plant surface; the more nutrients (sugars and amino acids) exuded from the plant, the more spores germinate and the faster they germinate. In some cases, spore germination of a certain pathogen is stimulated only by exudates of plants susceptible to that particular pathogen. In other cases, spore germination may be inhibited to a lesser or greater extent by materials

released into the surrounding water by the plant, by substances contained within the spores themselves, especially when the spores are highly concentrated ("quorum sensing"), and by saprophytic microflora present on or near the plant surface.

Fungi in soil coexist with a variety of antagonistic microorganisms that cause an environment of starvation and of toxic metabolites. As a result, spores of many soilborne fungi are often unable to germinate in some soils, and this phenomenon is called **fungistasis**, or their germ tubes lyse rapidly. Soils in which such events occur are known as **suppressive soils**. Fungistasis, however, is generally counteracted by root exudates of host plants growing nearby, and the spores are then able to germinate and infect.

After spores germinate, the resulting germ tube must grow, or the motile secondary spore (zoospore) must move, toward a site on the plant surface at which successful penetration can take place (Figs. 2-3A and 2-3B). The number, length, and rate of growth of germ tubes, or the number and mobility of motile spores, may be affected by physical conditions, such as temperature and moisture, by the kind and amount of exudates the plant produces at its surface, and by the saprophytic microflora.

The growth of germ tubes in the direction of successful penetration sites seems to be regulated by several factors, including greater humidity or chemical stimuli associated with such openings as wounds, stomata, and lenticels; thigmotropic (contact) responses to the topography of the leaf surface, resulting in germ tubes growing at right angles to cuticular ridges that generally surround stomata and thus eventually reaching a stoma; and nutritional responses of germ tubes toward greater concentrations of sugars and amino acids present along roots. The direction of movement of motile spores (zoospores) is also regulated by similar factors, namely chemical stimuli emanating from stomata, wounds, or the zone of elongation of roots, physical stimuli related to the structure of open stomata, and the nutrient gradient present in wound and root exudates.

Seeds germinate by producing a radicle, which either penetrates the host plant directly or first produces a small plant that subsequently penetrates the host plant by means of specialized feeding organs called haustoria. Most conditions described earlier as affecting spore germination and the direction of growth of germ tubes also apply to seeds. Haustoria are also produced by many fungi.

Hatching of Nematode Eggs

Nematode eggs also require conditions of favorable temperature and moisture to become activated and hatch. In most nematodes, the egg contains the first juvenile stage before or soon after the egg is laid. This juvenile immediately undergoes a molt and gives rise to the second juvenile stage, which may remain dormant in the egg for various periods of time. Thus, when the egg finally hatches, it is the second-stage juvenile that emerges, and it either finds and penetrates a host plant or undergoes additional molts that produce further juvenile stages and adults.

Once nematodes are in close proximity to plant roots, they are attracted to roots by certain chemical factors associated with root growth, particularly carbon dioxide and some amino acids. These factors may diffuse through soil and may have an attractant effect on nematodes present several centimeters away from the root. Nematodes are generally attracted to roots of both host and nonhost plants, although there may be some cases in which nematodes are attracted more strongly to the roots of host plants.

Penetration

Pathogens penetrate plant surfaces by direct penetration of cell walls, through natural openings, or through wounds (Figs. 2-3–2-5). Some fungi penetrate tissues in only one of these ways, others in more than one. Bacteria enter plants mostly through wounds, less frequently through natural openings, and never directly through unbroken cell walls (Fig. 2-5). Viruses, viroids, mollicutes, fastidious bacteria, and protozoa enter through wounds made by vectors, although some viruses and viroids may also enter through wounds made by tools and other means. Parasitic higher plants enter their hosts by direct penetration. Nematodes enter plants by direct penetration and, sometimes, through natural openings (Fig. 2-10).

Penetration does not always lead to infection. Many organisms actually penetrate cells of plants that are not susceptible to these organisms and that do not become diseased; these organisms cannot proceed beyond the stage of penetration and die without producing disease.

Direct Penetration through Intact Plant Surfaces

Direct penetration through intact plant surfaces is probably the most common type of penetration by fungi, oomycetes, and nematodes and the only type of penetration by parasitic higher plants. None of the other pathogens can enter plants by direct penetration.

Of the fungi that penetrate their host plants directly, the hemibiotrophic, i.e., nonobligate parasitic ones, do so through a fine hypha produced directly by the spore or mycelium (Figs. 2-3B, 2-5, and 2-8), whereas the

obligately parasitic ones do so through a penetration peg produced by an **appressorium** (Figs. 2-4B–2-4D and 2-9). The fine hypha or appressorium is formed at the point of contact of the germ tube or mycelium with a plant surface. The fine hypha grows toward the plant surface and pierces the cuticle and the cell wall through mechanical force and enzymatic softening of the cell wall substances. Most fungi, however, form an appressorium at the end of the germ tube, with the appressorium usually being bulbous or cylindrical with a flat surface in contact with the surface of the host plant (Figs. 2-4, 2-9Ab, and 2-9B). Then, a **penetration peg** grows from the flat surface of the appressorium toward the host and pierces the cuticle and the cell wall. The penetration peg grows into a fine hypha generally much smaller in diameter than a normal hypha of the fungus, but it regains its normal diameter once inside the cell. In most fungal diseases the fungus penetrates the plant cuticle and the cell wall, but in some, such as apple scab (Fig. 2-11A), the fungus penetrates only the cuticle and stays between the cuticle and the cell wall.

Parasitic higher plants also form an appressorium and penetration peg at the point of contact of the radicle with the host plant, and penetration is similar to that in fungi. Direct penetration in nematodes is accomplished by repeated back-and-forth thrusts of their stylets. Such thrusts finally create a small opening in the cell wall; the nematode then inserts its stylet into the cell or the entire nematode enters the cell (Fig. 2-12).

Penetration through Wounds

All bacteria, most fungi, some viruses, and all viroids can enter plants through various types of wounds (Fig. 2-5). Some viruses and all mollicutes, fastidious vascular bacteria, and protozoa enter plants through wounds made by their vectors. The wounds utilized by bacteria and fungi may be fresh or old and may consist of lacerated or killed tissue. These pathogens may grow briefly on such tissue before they advance into healthy tissue. Laceration or death of tissues may be the result of environmental factors such as wind breakage and hail; animal feeding, e.g., by insects and large animals; cultural practices of humans, such as pruning, transplanting, and harvesting; self-inflicted injuries, such as leaf scars; and, finally, wounds or lesions caused by other pathogens. Bacteria and fungi penetrating through wounds germinate or multiply in the wound sap or in a film of rain or dew water present on the wound. Subsequently, the pathogen invades adjacent plant cells or it secretes enzymes and toxins that kill and macerate the nearby cells.

The penetration of viruses, mollicutes, fastidious bacteria, and protozoa through wounds depends on the deposition of these pathogens by their vectors in fresh wounds created at the time of inoculation. All four types of pathogens are transmitted by certain types of insects. Some viruses are also transmitted by certain nematodes, mites, and fungi. Some viruses and viroids are transmitted through wounds made by human hands and tools. In most cases, however, these pathogens are carried by one or a few kinds of specific vectors and can be inoculated successfully only when they are brought to the plant by these particular vectors.

Penetration through Natural Openings

Many fungi and bacteria enter plants through stomata, and some enter through hydathodes, nectarthodes, and lenticels (Figs. 2-3, 2-4, 2-5, and 2-7). Stomata are most numerous on the lower side of leaves. They measure about 10–20 by 5–8 μm and are open in the daytime but are more or less closed at night. Bacteria present in a film of water over a stoma and, if water soaking occurs, can swim through the stoma easily (Fig. 2-3D) and into the substomatal cavity where they can multiply and start infection. Fungal spores generally germinate on the plant surface, and the germ tube may then grow through the stoma (Figs. 2-3A, 2-4B, and 2-5). Frequently, however, the germ tube forms an appressorium that fits tightly over the stoma, and usually one fine hypha grows from it into the stoma (Figs. 2-4 and 2-5). In the substomatal cavity the hypha enlarges, and from it grow one or several small hyphae that actually invade the cells of the host plant directly or by means of haustoria (Fig. 2-5). Although some fungi can apparently penetrate

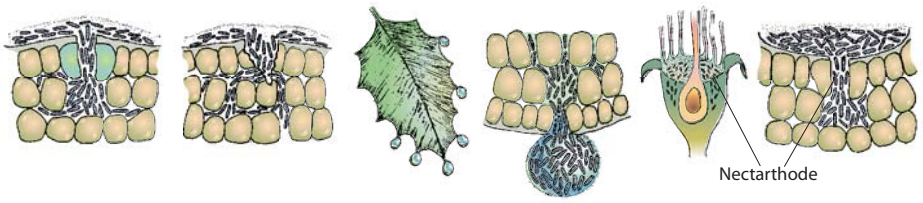


FIGURE 2-7 Methods of penetration and invasion by bacteria.

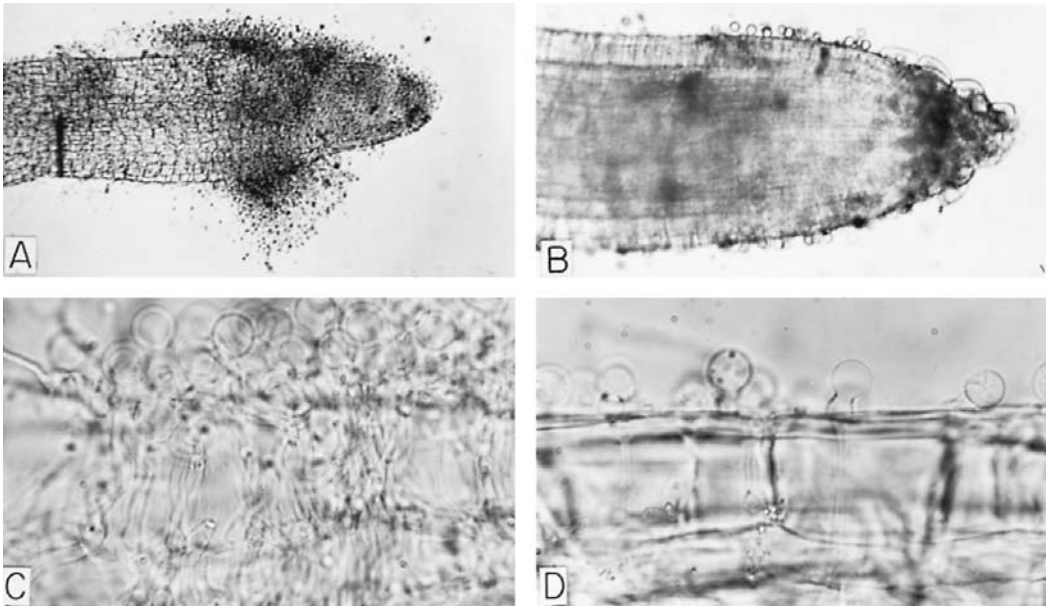


FIGURE 2-8 Attraction of zoospores of *Phytophthora cinnamomi* to roots of susceptible (A and C) and resistant (B and D) blueberry varieties, and infection of the roots by the zoospores. (A and B) Attraction of zoospores to roots 1 hour after inoculation. (C and D) Infection and colonization of the root after 24 hours are greater in the susceptible highbush blueberry (A and C) than in the more resistant rabbit-eye blueberry (B and D). (Photographs courtesy of R. D. Milholland.)

even closed stomata, others penetrate stomata only while they are open. Certain fungi, e.g., the powdery mildew fungi, may grow over open stomata without entering them.

Hydathodes are more or less permanently open pores at the margins and tips of leaves; they are connected to the veins and secrete droplets of liquid, called guttation drops, containing various nutrients (Fig. 2-5). Some bacteria use these pores as a means of entry into leaves, but few fungi seem to enter plants through hydathodes. Some bacteria also enter blossoms through the nectarthodes or nectaries, which are similar to hydathodes (Fig. 2-7).

Lenticels are openings on fruits, stems, and tubers that are filled with loosely connected cells that allow the passage of air. During the growing season, lenticels are open, but even so, relatively few fungi and bacteria penetrate tissues through them, growing and advancing mostly between the cells (Fig. 2-5). Most pathogens that penetrate through lenticels can also enter through wounds, with lenticel penetration being apparently a less efficient, secondary pathway.

Infection

Infection is the process by which pathogens establish contact with susceptible cells or tissues of the host and procure nutrients from them. Following infection, pathogens grow, multiply, or both within the plant tissues and invade and colonize the plant to a lesser or greater extent. Growth and/or reproduction of the pathogen (colonization) in or on infected tissues are actually two concurrent substages of disease development (Fig. 2-2).

Successful infections result in the appearance of symptoms, i.e., discolored, malformed, or necrotic areas on the host plant. Some infections, however, remain latent, i.e., they do not produce symptoms right away but at a later time when the environmental conditions or the stage of maturity of the plant become more favorable.

All the visible and otherwise detectable changes in the infected plants make up the **symptoms** of the disease. Symptoms may change continuously from the moment of their appearance until the entire plant dies or they may develop up to a point and then remain more or less unchanged for the rest of the growing season. Symptoms may appear as soon as 2 to 4 days after inoculation, as happens in some localized viral diseases of herbaceous plants, or as late as 2 to 3 years after inoculation, as in the case of some viral, mollicute, and other diseases of trees. In most plant diseases, however, symptoms appear from a few days to a few weeks after inoculation.

The time interval between inoculation and the appearance of disease symptoms is called the **incubation period**. The length of the incubation period of various diseases varies with the particular pathogen–host

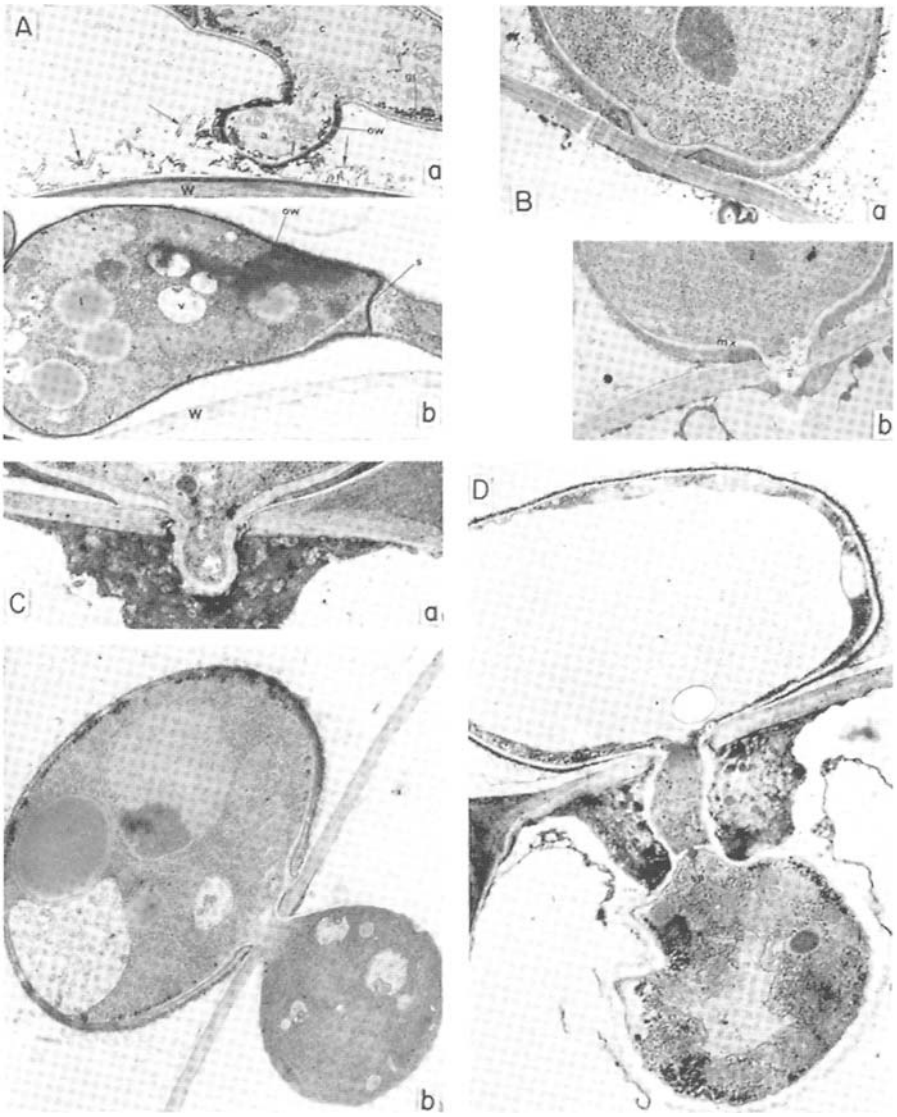
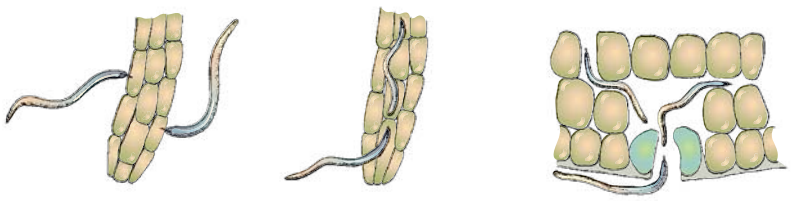


FIGURE 2-9 Electron micrographs of direct penetration of a fungus (*Colletotrichum graminicola*) into an epidermal leaf cell. (A) (a) Developing appressorium from a conidium. Note wax rods (arrows) on leaf surface. (b) Mature appressorium separated by a septum from the germination tube. (B) (a) Formation of penetration peg at the central point of contact of appressorium with the cell wall. (b) Structures in the penetration peg, which has already penetrated the cell wall, and papilla produced by the invaded cell. (C) Development of infection hypha. (a) Infection peg penetrating the papilla. (b) Appressorium and swollen infection hypha after penetration. (D) On completion of penetration and establishment of infection, the appressorium consists mostly of a large vacuole and is cut off from the infection hypha by a septum. (Photographs courtesy of D. J. Politis and H. Wheeler.)



Direct penetration
Ectoparasitic nematode

Direct penetration
Endoparasitic nematode

Penetration through stoma
Endoparasitic nematode

FIGURE 2-10 Methods of penetration and invasion by nematodes.

combination, with the stage of development of the host, and with the temperature in the environment of the infected plant.

During infection, some pathogens obtain nutrients from living cells, often without killing the cells or at least not for a long time; others kill cells and utilize their contents as they invade them; and still others kill cells and disorganize surrounding tissues. During infection, pathogens release a number of biologically active substances (e.g., enzymes, toxins, and growth regulators) that may affect the structural integrity of the host cells or their physiological processes. In response, the host reacts with a variety of defense mechanisms, which result in varying degrees of protection of the plant from the pathogen.

As mentioned earlier, for a successful infection to occur it is not sufficient that a pathogen comes in contact with its host; rather, several other conditions must also be satisfied. First of all, the plant variety must be susceptible to the particular pathogen and at a susceptible stage. The pathogen must be in a pathogenic stage that can infect immediately without requiring a resting (dormancy) period first, or infective juvenile stages or adults of nematodes. Finally, the temperature and moisture conditions in the environment of the plant must favor the growth and multiplication of the pathogen. When these conditions occur at an optimum, the pathogen can invade the host plant up to the maximum of its potential, even in the presence of plant defenses, and, as a consequence, disease develops.

Invasion

Various pathogens invade hosts in different ways and to different extents (Figs. 2-4, 2-5, 2-9, and 2-12). Some fungi, such as those causing apple scab and black spot of rose, produce mycelium that grows only in the area between the cuticle and the epidermis (subcuticular colonization) (Fig. 2-11A); others, such as those causing powdery mildews, produce mycelium only on the surface of the plant (Fig. 2-11B) but send haustoria into the epidermal cells. Most fungi spread into all the tissues of the plant organs (leaves, stems, and roots) they infect, either by growing directly through the cells as an **intracellular mycelium** or by growing between the cells as an **intercellular mycelium** (Figs. 2-11C and 2-11D). Fungi that cause vascular wilts invade the xylem vessels of plants (Fig. 2-11E).

Bacteria invade tissues intercellulary, although when parts of the cell walls dissolve, bacteria also grow intracellularly. Bacteria causing vascular wilts, like the vascular wilt fungi, invade the xylem vessels (Fig. 2-11E). Most nematodes invade tissues intercellularly, but some can invade intracellularly as well (Fig. 2-12). Many nematodes do not invade cells or tissues at all but feed by piercing epidermal cells with their stylets.

Viruses, viroids, mollicutes, fastidious bacteria, and protozoa invade tissues by moving from cell to cell intracellularly. Viruses and viroids invade all types of living plant cells, mollicutes and protozoa invade phloem sieve tubes and perhaps a few adjacent phloem parenchyma cells, and most fastidious bacteria invade xylem vessels and a few invade only phloem sieve tubes.

Many infections caused by fungi, bacteria, nematodes, viruses, and parasitic higher plants are local, i.e., they involve a single cell, a few cells, or a small area of the plant. These infections may remain localized throughout the growing season or they may enlarge slightly or very slowly. Other infections enlarge more or less rapidly and may involve an entire plant organ (flower, fruit, leaf), a large part of the plant (a branch), or the entire plant.

Infections caused by fastidious xylem- or phloem-inhabiting bacteria, mollicutes, and protozoa and natural infections caused by viruses and viroids are **systemic**, i.e., the pathogen, from one initial point in a plant, spreads and invades most or all susceptible cells and tissues throughout the plant. Vascular wilt fungi and bacteria invade xylem vessels internally, but they are usually confined to a few vessels in the roots, the stem, or the top of infected plants; only in the final stages of the disease do they invade most or all xylem vessels of the plant. Some downy mildew pathogens and some fungi, primarily among those causing smuts and rusts, also invade their hosts systemically, although in most cases the older mycelium degenerates and disappears and only the younger mycelium survives in actively growing plant tissues.

Growth and Reproduction of the Pathogen (Colonization)

Individual fungi and parasitic higher plants generally invade and infect tissues by growing on or into them from one initial point of inoculation. Most of these pathogens, whether inducing a small lesion, a large infected area, or a general necrosis of the plant, continue to grow and branch out within the infected host indefinitely so that the same pathogen individual spreads into more and more plant tissues until the spread of the infection is stopped or the plant is dead. In some fungal infections, however, while younger hyphae continue to grow into new healthy tissues, older ones in the already infected areas die out and disappear so that a diseased plant may have several points where separate units of the mycelium are active. Also, fungi causing vascular wilts often invade plants by producing and releasing spores within the vessels, and as the spores are carried

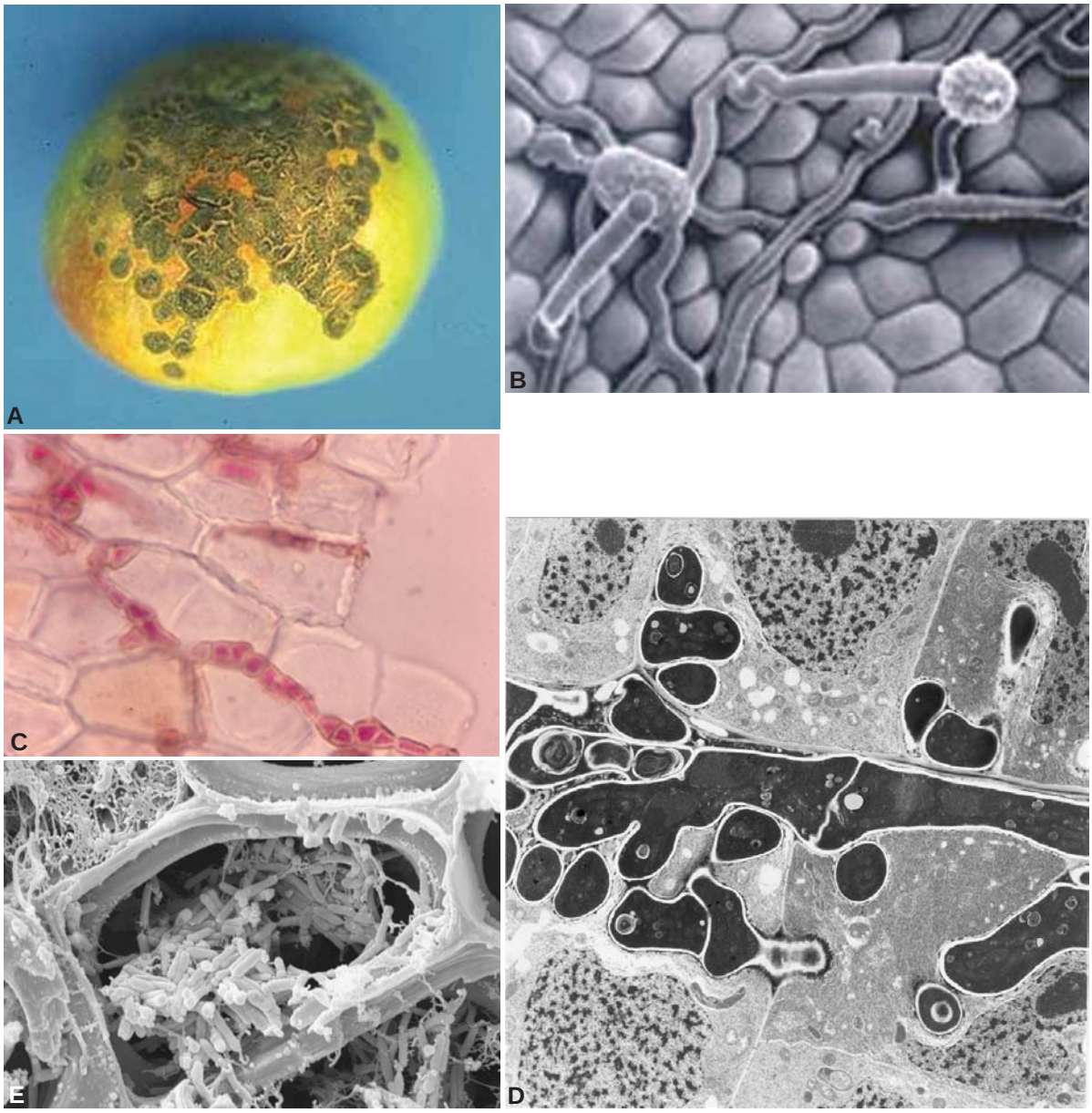


FIGURE 2-11 Types of invasion of pathogens in infected plants. (A) In apple scab disease, the pathogenic fungus grows only between the cuticle and the epidermal cells of leaves and fruit. (B) In powdery mildews the fungal mycelium grows only on the surface of host plants, but sends haustoria into the epidermal cells. (C) In many diseases the fungal mycelium (stained red here) grows only intercellularly (between the cells). (D) Hyphae of the smut fungus *Ustilago* in an infected leaf. (E) In bacterial vascular diseases, bacteria grow in and may clog the xylem vessels. [Photographs courtesy of (A) University of Oregon, (B) G. Celio, APS, (D) Mims *et al.* (1992). *Intern. J. Plant Sci.* **153**, 289–300, and (E) E. Alves, Federal University of Lavras, Brazil.]

in the sap stream they invade vessels far away from the mycelium, germinate there, and produce a mycelium, which invades more vessels.

All other pathogens, namely bacteria, mollicutes, viruses, viroids, nematodes, and protozoa, do not increase much, if at all, in size with time, as their size and shape remain relatively unchanged throughout their existence. These pathogens invade and infect new tissues within the plant by reproducing at a rapid rate and increasing their numbers tremendously in the infected tissues. The progeny may then be carried passively into new cells and tissues through plasmodesmata (viruses and viroids only), phloem (viruses, viroids, mollicutes, some fastidious bacteria, protozoa), or xylem (some bacteria); alternatively, as happens with protozoa and nematodes (Fig. 2-12) and somewhat with bacteria, they may move through cells on their own power.

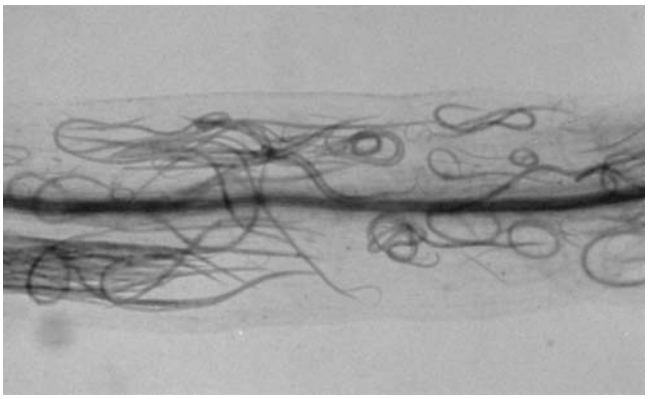


FIGURE 2-12 Alfalfa shoot invaded by plant parasitic nematodes (*Ditylenchus dipsaci*). (Photograph courtesy of J. Santo.)

Plant pathogens reproduce in a variety of ways (see Fig. 1-3 in Chapter 1). Fungi reproduce by means of spores, which may be either asexual (**mitospores**, i.e., products of mitosis, roughly equivalent to the buds on a twig or the tubers of a potato plant), or sexual (**meiospores**, i.e. products of meiosis, roughly equivalent to the seeds of plants). Parasitic higher plants reproduce just like all plants, i.e., by seeds. Bacteria and mollicutes reproduce by fission in which one mature individual splits into two equal, smaller individuals. Viruses and viroids are replicated by the cell, just as a page placed on a photocopying machine is replicated by the machine as long as the machine is operating and paper supplies last. Nematodes reproduce by means of eggs.

The great majority of plant pathogenic fungi and oomycetes produce a mycelium only within the plants they infect. Relatively few fungi and oomycetes produce a mycelium on the surface of their host plants, but most powdery mildew fungi produce a mycelium only on the surface of, and none within, their hosts (Figs. 2-13A–2-13C). The great majority of fungi and oomycetes

FIGURE 2-13 Means of reproduction of fungi and bacteria. (A–E) Mycelium [white material on leaf (A, B)], chains of conidia (C), and cleistothecium (B and D) (containing four asci, each containing ascospores) on the leaf surface. (E) Apple trees having numerous branches killed by the fire blight bacterium. (F) Large numbers of bacteria inside a xylem vessel of a bacterial wilt-infected plant. [Photographs courtesy of (A and B) D. Legard, University of Florida, (C) D. Mathre, Montana State University, (D) M. Hoffman, Oregon State University, (E) A. Jones, Michigan State University, and (F) B. Bruton, USDA, Lane, Oklahoma.]

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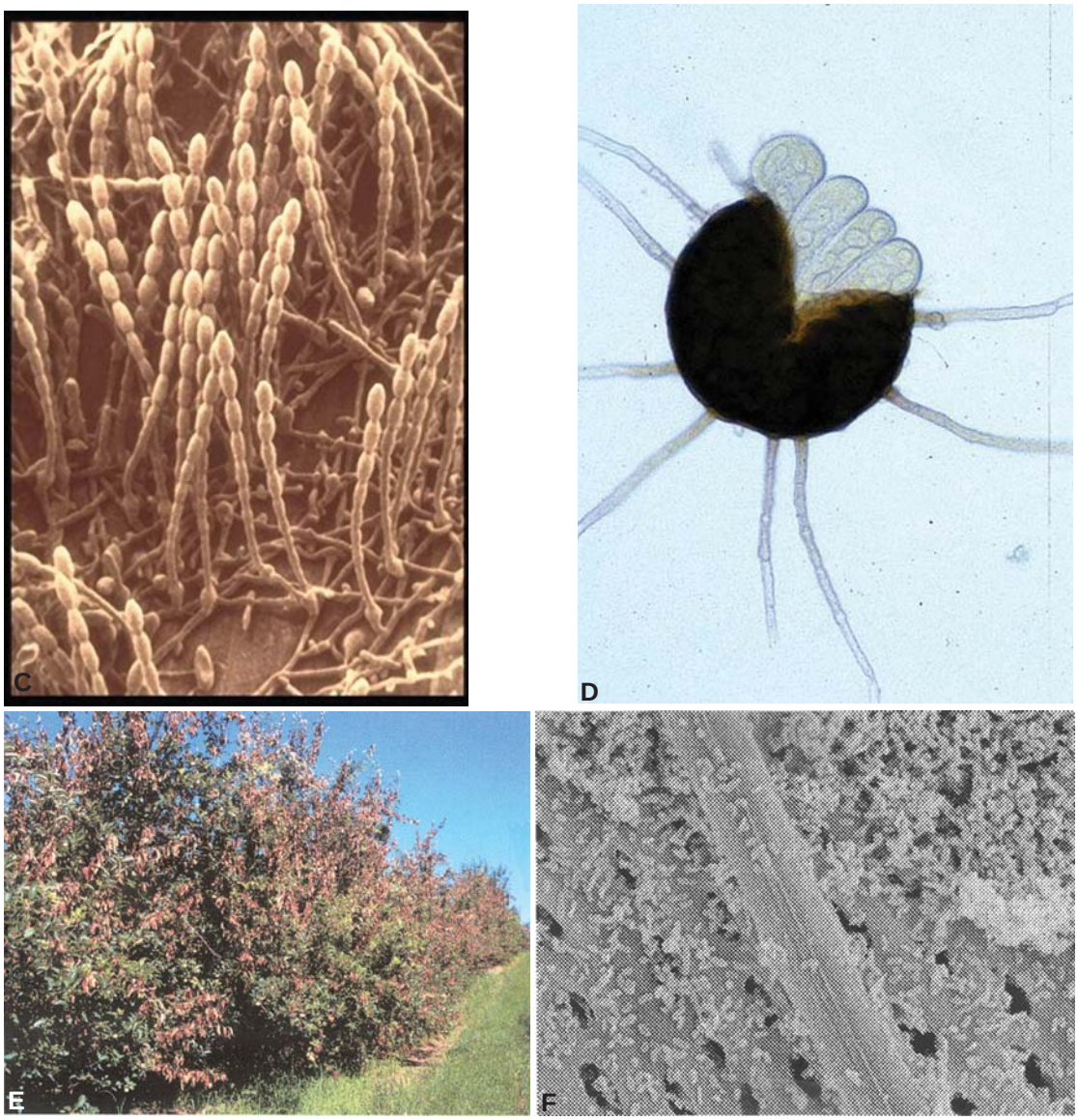

FIGURE 2-13 (*Continued*)

produce spores on, or just below, the surface of the infected area of the host, and the spores are released outward into the environment. Plant pathogenic plasmodiophoromycetes, however, such as the clubroot pathogen and fungi causing vascular wilts, produce spores within the host tissues, and these spores are not released outward until the host dies and disintegrates. Parasitic higher plants produce their seeds on aerial branches, and some nematodes lay their eggs at or near the surface of the host plant. Bacteria reproduce between or, in xylem- or phloem-inhabiting bacteria, within host cells (Fig. 2-13F), generally inside the host plant; they come to the host surface only through wounds, cracks, stomata, and so on. Viruses, viroids, mollicutes, protozoa, and fastidious bacteria reproduce only inside cells and apparently do not reach or exist on the surface of the host plant.

The rate of reproduction varies considerably among the various kinds of pathogens, but in all types, one or a few pathogens can produce tremendous numbers of individuals within one growing season. Some fungi produce spores more or less continuously (Fig. 2-14), whereas others produce them in successive crops. In either case, several thousand to several hundreds of

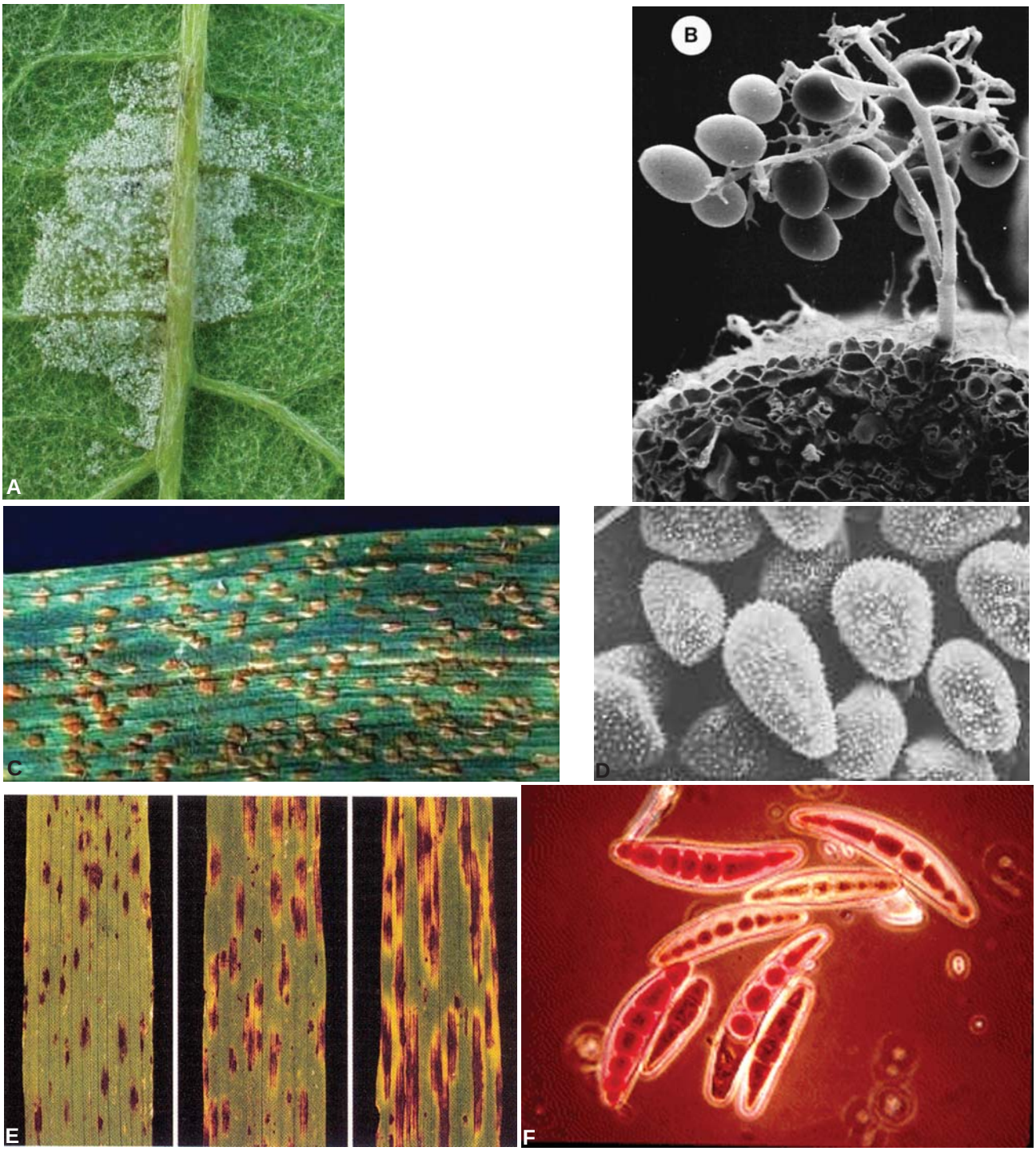

FIGURE 2-14 Invasion and reproduction of oomycete and fungal plant pathogens. Sporangiophores and sporangia (A) on the underside of a grape leaf infected with the grape downy mildew pathogen *Plasmopara viticola* and (B) on the root of a lettuce plant infected with *Plasmopara lactucae-radicis*. (C) A wheat leaf showing numerous infection lesions (uredia) of the leaf rust fungus. (D) Uredospores of the soybean rust. (E) Leaves of three barley varieties showing infection lesions, the severity (number and size) of which are inversely proportional to the degree of resistance of each variety to the fungal pathogen. (F) Spores of the fungus *Cochliobolus* that cause leaf spot on barley. [Photographs courtesy of (A) J. Rytter and J. W. Travis, Pennsylvania State University, (B) M. E. Stanghellini, University of California, Riverside, and (E) B. Steffenson, University of Minnesota.]

thousands of spores may be produced per square centimeter of infected tissue. Even small specialized sporophores can produce millions of spores, and the number of spores produced per diseased plant is often in the billions or trillions (Fig. 2-14). The number of spores produced in an acre of heavily infected plants, therefore, is generally astronomical, and enough spores are released to land on every conceivable surface in the field and the surrounding areas, enough to easily inoculate with a heavy inoculum every plant in the area.

Bacteria reproduce rapidly within infected tissues (Fig. 2-13F). Under optimum nutritional and environmental conditions (in culture), bacteria divide (double their numbers) every 20 to 30 minutes, and, presumably, bacteria multiply just as fast in a susceptible plant as long as nutrients and space are available and the temperature is favorable. Millions of bacteria may be present in a single drop of infected plant sap so the number of bacteria per plant must be astronomical. Fastidious bacteria and mollicutes appear to reproduce more slowly than typical bacteria; although they spread systemically throughout the vascular system of the plant, they are present in relatively few xylem or phloem vessels, and the total number of these pathogens in infected plants is relatively small. This also seems to be true for protozoa.

Viruses and viroids reproduce within living host cells, with the first new virus particles being detectable several hours after infection. Soon after that, however, virus particles accumulate within the infected living cell until as many as 100,000 to 10,000,000 particles may be present in a single cell. Viruses and viroids infect and multiply in most or all living cells of their hosts, and it is apparent that each plant may contain innumerable individuals of these pathogens.

Nematode females lay about 300 to 500 eggs, about half of which produce females that again lay 300 to 600 eggs each. Depending on the climate, the availability of hosts, and the duration of each life cycle of the particular nematode, a nematode species may have from two to more than a dozen generations per year. If even just half of the females survived and reproduced, each generation time would increase the number of nematodes in the soil by more than a hundred fold. Thus, the buildup of nematode populations within a growing season and in successive seasons is often quite dramatic.

Dissemination of the Pathogen

A few pathogens, such as nematodes, oomycetes, zoosporic fungi, and bacteria, can move short distances on their own power and thus can move from one host to another one very close to it. Fungal hyphae can grow between tissues in contact and sometimes through the soil toward nearby roots for a few to many centimeters. Both of these means of dissemination, however, are quite limited, especially in the case of zoospores and bacteria.

The spores of some fungi are expelled forcibly from the sporophore or sporocarp by a squirting or puffing action that results in the successive or simultaneous discharge of spores up to a centimeter or so above the sporophore. The seeds of some parasitic plants are also expelled forcibly and may arch over distances of several meters.

Almost all dissemination of pathogens responsible for plant disease outbreaks, and even for disease occurrences of minor economic importance, is carried out passively by such agents as air and insects (Figs. 2-13–2-15). To a lesser extent, water, certain other animals, and humans may be involved (Fig. 2-15).

Dissemination by Air

Spores of most oomycetes and most fungi and the seeds of most parasitic plants are disseminated by air currents that carry them as inert particles to various distances. Air currents pick up spores and seeds off the sporophores (Figs. 2-13A–2-13E, 2-14, and 2-16) or while they are being expelled forcibly or are falling at maturity. Depending on the air turbulence and velocity, air currents may carry the spores upward or horizontally in a way similar to that of particles contained in smoke. While airborne, some of the spores may touch wet surfaces and get trapped; when air movement stops or when it rains, the rest of the spores land or are "washed out" from the air and are brought down by the raindrops. Most of the spores, of course, land on anything but a susceptible host plant. Also, the spores of many fungi are actually too delicate to survive a long trip through the air and are therefore successfully disseminated through the air for only a few hundred or a few thousand meters. The spores of other fungi, however, particularly those of the cereal rusts, are very hardy and occur commonly at all levels and at high altitudes (several thousand meters) above infected fields. Spores of these fungi are often carried over distances of several kilometers, even hundreds of kilometers, and in favorable weather may cause widespread epidemics. Some fungi can spread into new areas quite rapidly and may cause severe epidemics over large areas, including entire continents, within a few years. This happened, for example, in the airborne pathogens of sugar cane smut in the Americas (Fig. 2-18) and of barley stripe rust in South America (Fig. 2-15).

Air dissemination of other pathogens occurs rather infrequently and only under special conditions, or indi-

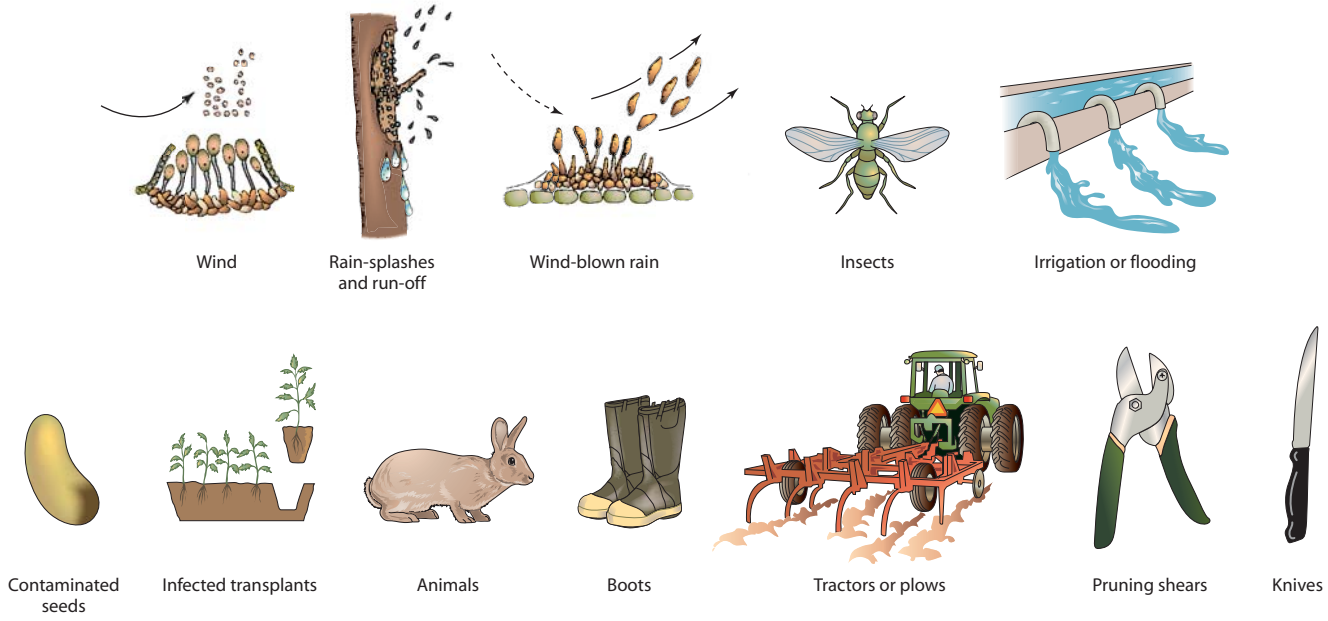

FIGURE 2-15 Means of dissemination of fungi and bacteria.

rectly. For example, bacteria causing fire blight of apple and pear produce fine strands of dried bacterial exudate containing bacteria, and these strands may be broken off and disseminated by wind. Bacteria and nematodes present in the soil may be blown away along with plant debris or soil particles in the dust. Wind also helps in the dissemination of bacteria, fungal spores, and nematodes by blowing away rain splash droplets containing these pathogens, and wind carries away insects that may contain or are smeared with viruses, bacteria, mollicutes, protozoa, or fungal spores. Finally, wind causes adjacent plants or plant parts to rub against one another, which may help the spread by contact of bacteria, fungi, some viruses and viroids, and possibly some nematodes.

Dissemination by Water

Water is important in disseminating pathogens in three ways. (1) Bacteria, nematodes, and spores and mycelial fragments of fungi present in the soil are disseminated by rain or irrigation water that moves on the surface or through the soil. (2) All bacteria and the spores of many fungi are exuded in a sticky liquid (Figs. 2-16A, 2-16B, and 2-16D) and depend on rain or (overhead) irrigation water, which either washes them downward or splashes them in all directions, for their dissemination (3) Raindrops or drops from overhead irrigation pick up the fungal spores and any bacteria present in the air and wash them downward, where some of them may land on susceptible plants. Although water is less important than air in the long-distance transport of pathogens, the water dissemination of pathogens is more efficient for nearby infections, as the pathogens land on an already wet surface and can move or germinate immediately.

Dissemination by Insects, Mites, Nematodes, and Other Vectors

Insects, particularly aphids, leafhoppers, and whiteflies, are by far the most important vectors of viruses, whereas leafhoppers are the main vectors of mollicutes, fastidious bacteria, and protozoa. Each one of these pathogens is transmitted, internally, by only one or a few species of insects during feeding and movement of the insect vectors from plant to plant. Specific insects also transmit certain fungal, bacterial, and nematode pathogens, such as the fungus causing Dutch elm disease, the bacterial wilt of cucurbits, and the pine wilt nematode. In all diseases in which the pathogen is carried internally or externally by one or a few specific vectors, dissemination of the pathogen depends, to a large extent or entirely, on that vector. In many diseases, however, such as bacterial soft rots, fungal fruit rots, anthracnoses, and ergot, insects become smeared with various kinds of bacteria or sticky fungal spores as they move among plants. The insects carry these pathogens externally from plant to plant and deposit them on the plant surface or in the wounds they make on the plants during feeding. In such diseases, dissemination of the pathogen is facilitated by but is not dependent on the vector. Insects may disseminate pathogens over short or long


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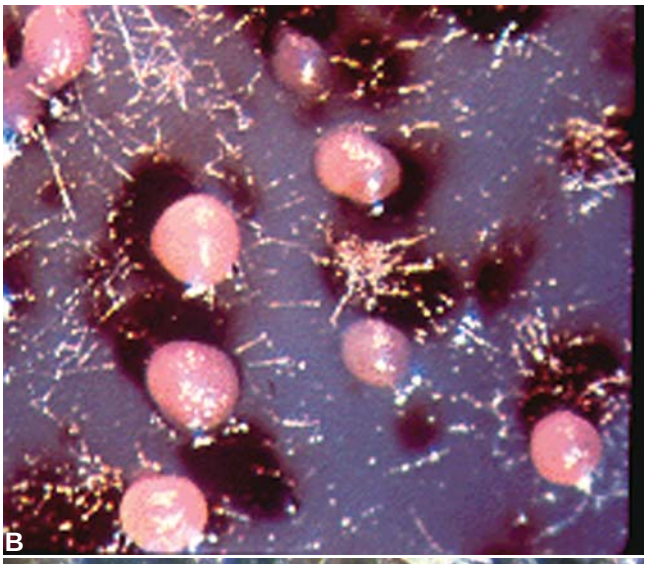
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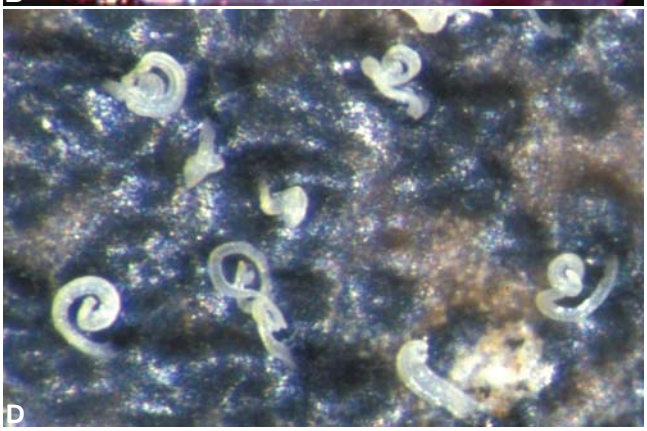
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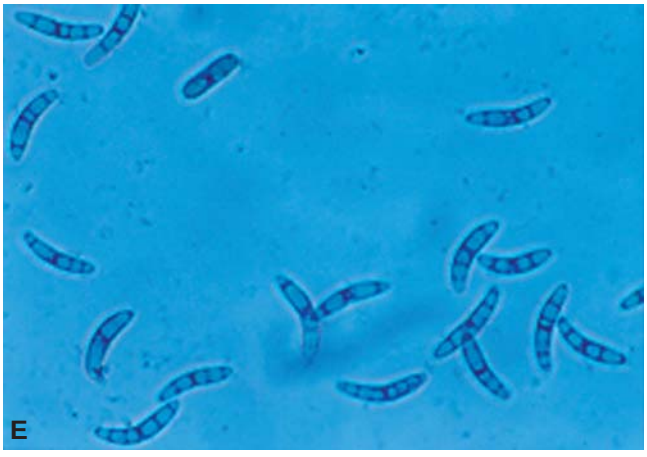
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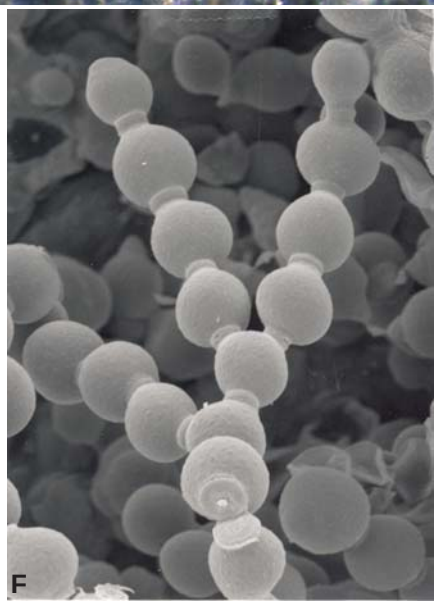
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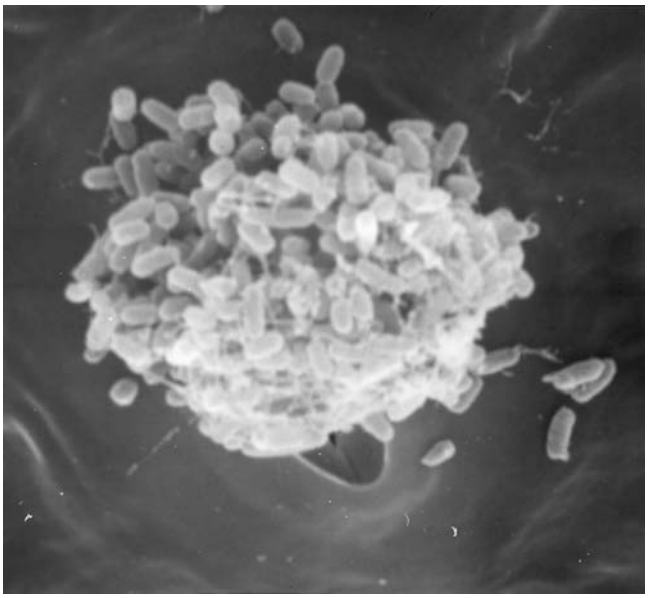

FIGURE 2-17 *Pseudomonas syringae* bacteria exuding through the stoma of an infected cherry leaf (2500X). (Photograph courtesy of E. L. Mansvelt, Stellenbosch, South Africa.)

distances, depending on the kind of insect, the insect–pathogen association, and the prevailing weather conditions, particularly wind.

A few species of mites and nematodes can transmit internally several viruses from plant to plant. In addition, mites and nematodes probably carry externally bacteria and sticky fungal spores with which they become smeared as they move on infected plant surfaces.

Almost all animals, small and large, that move among plants and touch the plants along the way can disseminate pathogens such as fungal spores, bacteria, seeds of parasitic plants, nematodes, and perhaps some viruses and viroids. Most of these pathogens adhere to the feet or the body of the animals, but some may be carried in contaminated mouthparts.

Finally, some plant pathogens, e.g., the zoospores of some fungi and certain parasitic plants, can transmit viruses as they move from one plant to another (zoospores) or as they grow and form a bridge between two plants (dodder).

FIGURE 2-18 Map of the rapid spread of sugarcane smut, caused by the fungus *Ustilago scitaminea*, from its first sighting in Guyana in 1974 throughout the Caribbean islands, Central America, and the United States by 1981. [From Comstock *et al.* (1983). *Plant Dis.* **67**, 452–457.]

FIGURE 2-16 Fungal spore production, overwintering, and dissemination. (A) Pycnidia containing conidia produced on the stem of an infected plant. (B) Conidia oozing out of pycnidia after the latter absorbed rainwater. (C) Pile of cull potatoes in which many pathogens, such as the late blight oomycete, *Phytophthora infestans*, overwinter and are subsequently carried from the cull piles to potato fields. (D) Tendrils of conidia produced from hydrated bark-embedded pycnidia of the apple white rot fungus, *Botryosphaeria obtusa*. (E) Spores of the canker-causing fungus *Nectria*. (F) Chains of conidia of *Monilinia* sp. [Photographs courtesy of (A, B, and E) R. Cullen, University of Florida, (C) Plant Pathology Department, University of Wisconsin, (D) J. Rytter and J. W. Travis, Pennsylvania State University, and (F) and Mims *et al.* (1999). *Mycologia* **91**, 499–509.]

FIGURE 2-19 Map of the spatial and temporal spread of barley stripe rust, caused by the fungus *Puccinia striiformis f. sp. hordei*, in South America. The sequence of sightings are 1, Colombia 1975; 2, Ecuador 1976; 3, Peru 1977; 4, Bolivia 1978; 5, Chile 1980; and 6, Argentina 1982. [From Dubin and Stubbs (1986). *Plant Dis.* 70, 141–144.]

Dissemination by Pollen, Seed, Transplants, Budwood, and Nursery Stock

Some viruses are carried in the pollen of plants infected with these viruses and, when virus-carrying pollen pollinates a healthy plant, the virus may infect not only the seed produced from such pollination, which will then grow into a virus-infected plant, it may also infect the plant that was pollinated with the virus-carrying pollen.

Many pathogens are present on or in seeds, transplants, budwood, or nursery stock and are disseminated by them as the latter are transported to other fields or are sold and transported to other areas near and far. Dissemination of pathogens through seed, transplants, and so on is of great practical importance because it introduces the pathogen along with the plant at the beginning of the growth season and enables the pathogen to multiply and be disseminated by all the other means of spread discussed. It is also important because it brings pathogens into new areas where they may have never existed before.

Dissemination by Humans

Human beings disseminate all kinds of pathogens over short and long distances in a variety of ways. Within a field, humans disseminate some pathogens, such as tobacco mosaic virus, through the successive handling of diseased and healthy plants. Other pathogens are disseminated through tools, such as pruning shears, contaminated when used on diseased plants (e.g., pear infected with fire blight bacteria), and then carried to healthy plants. Humans also disseminate pathogens by transporting contaminated soil on their feet or equipment, using contaminated containers, and using infected transplants, seed, nursery stock, and budwood as mentioned previously. Finally, humans disseminate pathogens by importing new varieties into an area that may carry pathogens that have gone undetected, by traveling throughout the world, and by importing food or other items that may carry harmful plant pathogens. Examples of the role of humans as a vector of pathogens can be seen in the introduction into the United States of the fungi causing Dutch elm disease and white pine blister rust and of the citrus canker bacterium, in the introduction in Europe of the powdery and downy mildews of grape, and, more recently, in the rapid spread of sorghum ergot almost throughout the world (Fig. 2-20).

Overwintering and/or Oversummering of Pathogens

Pathogens that infect perennial plants can survive in them during low winter temperatures, during the hot, dry weather of the summer, or both, regardless of whether the host plants are actively growing or are dormant at the time. Annual plants, however, die at the end of the growing season, as do the leaves and fruits of deciduous perennial plants and even the stems of some perennial plants. In colder climates, annual plants and the tops of some perennial plants die with the advent of low winter temperatures, and their pathogens are left without a host for the several months of cold weather. In hot, dry climates, however, annual plants die during the summer and their pathogens must be able to survive such periods in the absence of their hosts. Thus, pathogens that attack annual plants and renewable parts of perennial plants have evolved mechanisms by which they can survive the cold winters or dry summers that may intervene between crops or growing seasons (Fig. 2-21).

Fungi have evolved a great variety of mechanisms for persisting between crops. On perennial plants, fungi overwinter as mycelium in diseased tissues, e.g., cankers,

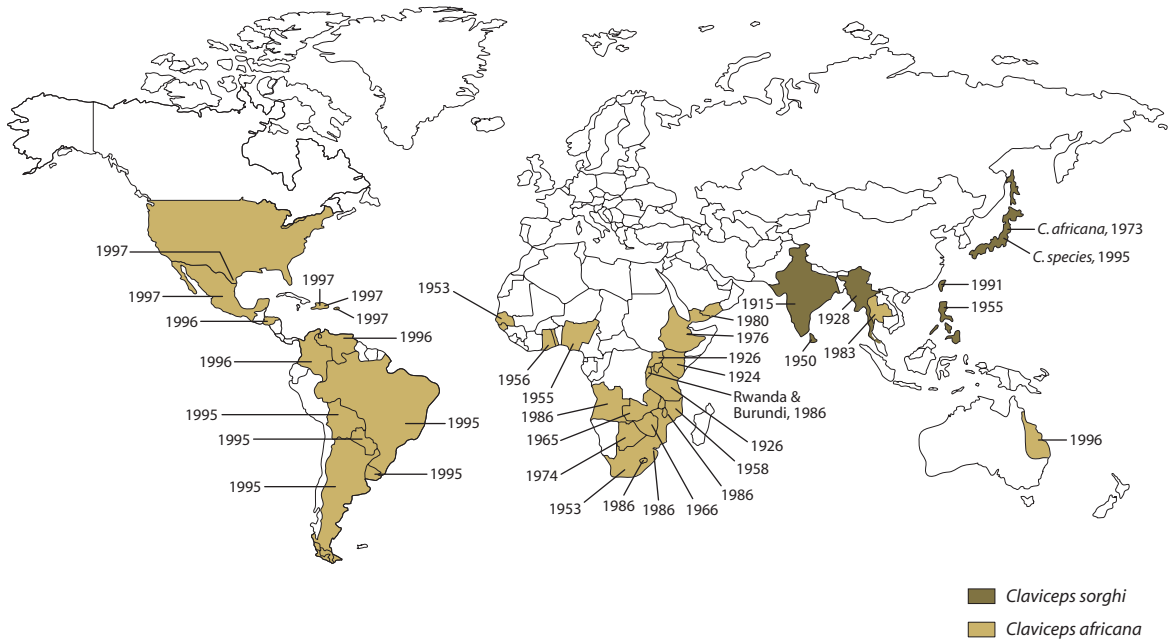

FIGURE 2-20 Map of the history of spread of ergot of sorghum, caused primarily by the fungus *Claviceps africana*, around the world. [Photograph courtesy of R. Bandyopadhyay *et al.* (1998). *Plant Dis.* **82,** 356–367.]

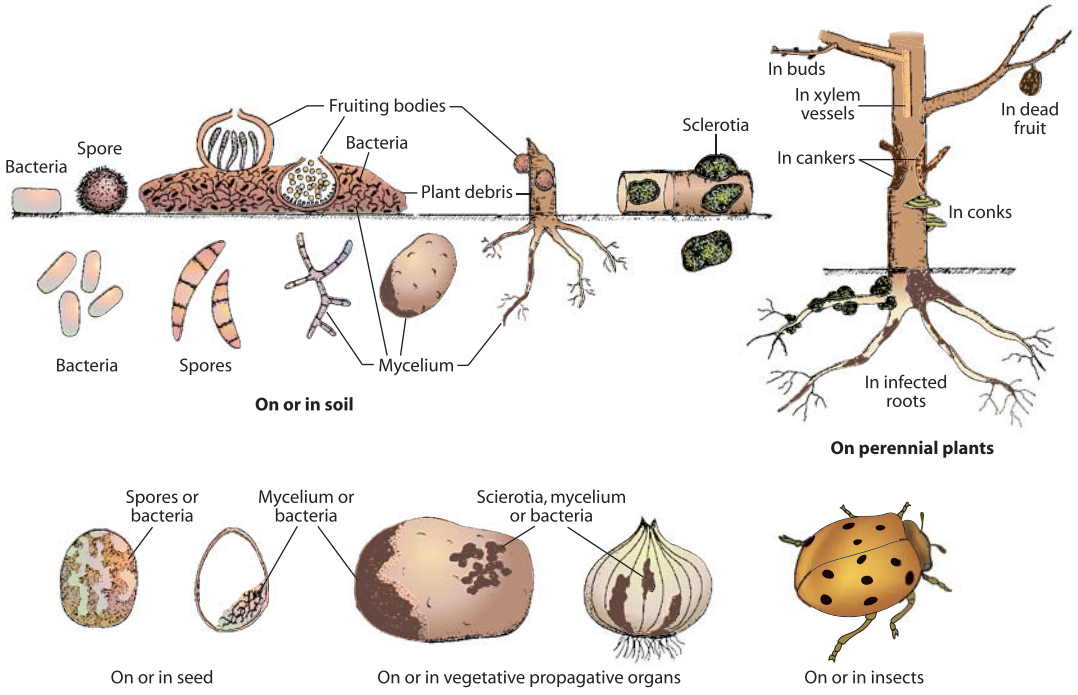

FIGURE 2-21 Forms and locations of survival of fungi and bacteria between crops.

and as spores at or near the infected surface of the plant or on the bud scales. Fungi affecting leaves or fruits of deciduous trees usually overwinter as mycelium or spores on fallen, infected leaves or fruits or on the bud scales. Fungi affecting annual plants usually survive the winter or summer as mycelium in infected plant debris, as resting or other spores and as sclerotia (hard masses of mycelium) in infected plant debris or in the soil, and as mycelium, spores, or sclerotia in or on seeds and other propagative organs, such as tubers. Some plant pathogenic oomycetes (e.g., *Pythium*) and fungi (e.g., *Fusarium, Rhizoctonia*) are **soil inhabitants**, i.e., they are able to survive indefinitely as saprophytes. Soil inhabitants are generally unspecialized parasites that have a wide host range. Other fungi are **soil transients**, i.e., they are rather specialized parasites that generally live in close association with their host but may survive in the soil for relatively short periods of time as hardy spores or as saprophytes. In some areas, fungi survive by continuous infection of host plants grown outdoors throughout the year, such as cabbage, or of plants grown in the greenhouse in the winter and outdoors in the summer. Similarly, some rust and other fungi overwinter on winter crops grown in warmer climates and move from them to the same hosts grown as spring crops in colder climates. Also, some fungi infect cultivated or wild perennial, as well as annual, plants and move from the perennial to the annual ones each growth season. Some rust fungi infect alternately an annual and a perennial host, and the fungus goes from the one to the other host and overwinters in the perennial host.

Bacteria overwinter and oversummer as bacteria in essentially the same ways as described for fungi, i.e., in infected plants, seeds, and tubers, in infected plant debris, and, for some, in the soil. Bacteria survive poorly when present in small numbers and free in the soil but survive well when masses of them are embedded in the hardened, slimy polysaccharides that usually surround them. Some bacteria also overwinter within the bodies of their insect vectors.

Viruses, viroids, mollicutes, fastidious bacteria, and protozoa survive only in living plant tissues such as the tops and roots of perennial plants, the roots of perennial plants that die to the soil line in the winter or summer, vegetative propagating organs, and the seeds of some hosts. A few viruses survive within their insect vectors, and some viruses and viroids may survive on contaminated tools and in infected plant debris.

Nematodes usually overwinter or oversummer as eggs in the soil and as eggs or nematodes in plant roots or in plant debris. Some nematodes produce juvenile stages or adults that can remain dormant in seeds or on bulbs for many months or years. Finally, parasitic higher plants survive either as seeds, usually in the soil, or as their infective vegetative form on their host.

RELATIONSHIPS BETWEEN DISEASE CYCLES AND EPIDEMICS

Some pathogens complete only one, or even part of one, disease cycle in 1 year and are called monocyclic, or single-cycle, pathogens (Fig. 2-22). Diseases caused by monocyclic pathogens include the smuts, in which the fungus produces spores at the end of the season (these spores serve as primary — and only — inoculum for the following year); many tree rusts, which require two alternate hosts and at least 1 year to complete one disease cycle; and many soilborne diseases, e.g., root rots and vascular wilts. In root rots and vascular wilts, the pathogens survive the winter or summer in decaying stems and roots or in the soil, infect plants during the growth season, and, at the end of the growth season, produce new spores in the infected stems and roots. These spores remain in the soil and serve as the primary inoculum the following growth season. In monocyclic pathogens the primary inoculum is the only inoculum available for the entire season, as there is no secondary inoculum and no secondary infection. The amount of inoculum produced at the end of the season, however, is greater than that present at the start of the season and so in monocyclic diseases the amount of inoculum may increase steadily from year to year.

In most diseases, however, the pathogen goes through more than one generation per growth season, and such pathogens are called polycyclic, or multicyclic, pathogens (Fig. 2-22). Polycyclic pathogens can complete many (from 2 to 30) disease cycles per year, and with each cycle the amount of inoculum is multiplied manyfold. Polycyclic pathogens are disseminated primarily by air or airborne vectors (insects) and are responsible for the kinds of diseases that cause most of

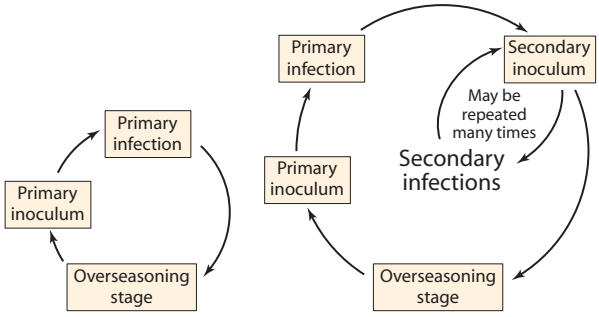

FIGURE 2-22 Diagrams of (left) monocyclic and (right) polycyclic plant diseases. Monocyclic diseases lack secondary inoculum and secondary infections during the same year.

the explosive epidemics on most crops, e.g., downy mildews, late blight of potato, powdery mildews, leaf spots and blights, grain rusts, and insect-borne viruses. In polycyclic fungal pathogens, the primary inoculum often consists of the sexual (perfect) spore or, in fungi that lack the sexual stage, some other hardy structure of the fungus such as sclerotia, pseudosclerotia, or mycelium in infected tissue. The number of sexual spores or other hardy structures that survive and cause infection is usually small, but once primary infection takes place, large numbers of asexual spores (secondary inoculum) are produced at each infection site and these spores can themselves cause new (secondary) infections that produce more asexual spores for more infections.

In some diseases of trees, e.g., fungal vascular wilts, phytoplasmal declines, and viral infections, the infecting pathogen may not complete a disease cycle, i.e., it may not produce inoculum that can be disseminated and initiate new infections, until at least the following year and some may take longer. Such diseases are basically monocyclic, but if they take more than a year to complete the cycle, they are called polyetic (multiyear). There are pathogens, however, such as those causing several rusts of trees and the mistletoes, that take several years to go through all the stages of their life cycle and to initiate new infections. These pathogens and the diseases they cause are clearly polyetic. Although polyetic pathogens may not cause many new infections over a given area within a single year and their amount of inoculum does not increase greatly within a year, because they survive in perennial hosts they have the advantage that, at the start of each year, they have almost as much inoculum as they had at the end of the previous year. Therefore, the inoculum may increase steadily (exponentially) from year to year and may cause severe epidemics when considered over several years. Examples of such diseases are Dutch elm disease, cedar apple rust, white pine blister rust, and citrus tristeza.

Whether the pathogen involved in a particular disease is monocyclic, polycyclic, or polyetic has great epidemiological consequences because it affects the amount of disease caused by the specific pathogen within a given period of time. The rate of inoculum or disease increase (r) has been calculated for many diseases and varies from 0.1 to 0.5 per day for polycyclic foliar diseases, such as southern corn leaf blight, potato late blight, grain rusts, and tobacco mosaic, to 0.02 to 2.3 per year for polyetic diseases of trees such as dwarf mistletoe of conifers, Dutch elm disease, chestnut blight, and peach mosaic. These values of r signify an increase in the amount of inoculum or disease (number of plants infected, amount of plant tissue infected, and so on) from 10 to 50% per day for polycyclic foliar diseases and from 2 to 230% per year for polyetic diseases of trees such as those listed earlier.

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chapter three

EFFECTS *OF* PATHOGENS *ON* PLANT PHYSIOLOGICAL FUNCTIONS

INTRODUCTION

While pathogens infect plants in the course of their obtaining food for themselves, depending on the kind of pathogen and on the plant organ and tissue they infect, pathogens interfere with the different physiological function(s) of the plant and lead to the development of different symptoms. Thus, a pathogen that infects and kills the flowers of a plant interferes with the ability of the plant to produce seed and multiply. A pathogen that infects and kills part or all of the roots of a plant reduces the ability of the plant to absorb

water and nutrients and results in its wilting and death. Similarly, a pathogen that infects and kills parts of the leaves or destroys their chlorophyll leads to reduced photosynthesis, growth, and yield of the plant, and so forth. In most cases the relationship between the symptoms of the plant and the physiological functions affected is obvious and understandable. In other cases, however, the relationship of the two is more complex and the explanation is not always straightforward.

EFFECT OF PATHOGENS ON PHOTOSYNTHESIS

Photosynthesis is the basic function of green plants: it enables them to transform light energy into chemical energy, which they can utilize in all cell activities. Photosynthesis is the ultimate source of nearly all energy used in all living cells, plant or animal, as all activities of living cells, except photosynthesis, expend the energy provided by photosynthesis. In photosynthesis, carbon dioxide from the atmosphere and water from the soil are brought together in the chloroplasts of the green parts of plants and, in the presence of light, react to form glucose with a concurrent release of oxygen:

$$6CO_2 + 6H_2O \xrightarrow[\text{chlorophyll}]{\text{light}} C_6H_{12}O_6 + 6O_2$$

In view of the fundamental position of photosynthesis in the life of plants, it is apparent that any interference by pathogens with photosynthesis results in a diseased condition in the plant. That pathogens do interfere with photosynthesis is obvious from the chlorosis they cause on many infected plants, from the necrotic lesions or large necrotic areas they produce on green plant parts, and from the reduced growth and amounts of fruits produced by many infected plants.

In leaf spot, blight, and other kinds of diseases in which there is destruction of leaf tissue, e.g., in cereal rusts and fungal leaf spots (Figs. 3-1A–3-1C), bacterial leaf spots (Fig. 3-1D), viral mosaics (Fig. 3-1E) and yellowing and stunting diseases (Fig. 3-1F), or in defoliations, photosynthesis is reduced because the photosynthetic surface of the plant is lessened. Even in other diseases, however, plant pathogens reduce photosynthesis, especially in the late stages of diseases, by affecting the chloroplasts and causing their degeneration. The overall chlorophyll content of leaves in many fungal and bacterial diseases is reduced, but the photosynthetic activity of the remaining chlorophyll seems to remain unaffected. In some fungal and bacterial diseases, photosynthesis is reduced because the toxins, such as tentoxin and tabtoxin, produced by these pathogens inhibit some of the enzymes that are involved directly or indirectly in photosynthesis. In plants infected by many vascular pathogens, stomata remain partially closed, chlorophyll is reduced, and photosynthesis stops even before the plant eventually wilts. Most virus, mollicute, and nematode diseases also induce varying degrees of chlorosis and stunting. In the majority of such diseases, the photosynthesis of infected plants is reduced greatly. In advanced stages of disease, the rate of photosynthesis is no more than one-fourth the normal rate.

EFFECT OF PATHOGENS ON TRANSLOCATION OF WATER AND NUTRIENTS IN THE HOST PLANT

All living plant cells require an abundance of water and an adequate amount of organic and inorganic nutrients in order to live and to carry out their physiological functions. Plants absorb water and inorganic (mineral) nutrients from the soil through their root system. These substances are generally translocated upward through the xylem vessels of the stem and into the vascular bundles of the petioles and leaf veins, from which they enter the leaf cells. Minerals and part of the water are utilized by the leaf and other cells for the synthesis of the various plant substances, but most of the water evaporates out of the leaf cells into the intercellular spaces and from there diffuses into the atmosphere through the stomata. However, nearly all organic nutrients of plants are produced in the leaf cells, following photosynthesis, and are translocated downward and distributed to all the living plant cells by passing, for the most part, through the phloem tissues. When a pathogen interferes with the upward movement of inorganic nutrients and water or with the downward movement of organic substances, diseased conditions result in the parts of the plant denied these materials. The diseased parts, in turn, will be unable to carry out their own functions and will deny the rest of the plant their services or their products, thus causing disease of the entire plant. For example, if water movement to the leaves is inhibited, the leaves cannot function properly, photosynthesis is reduced or stopped, and few or no nutrients are available to move to the roots, which in turn become starved and diseased and may die.

Interference with Upward Translocation of Water and Inorganic Nutrients

Many plant pathogens interfere in one or more ways with the translocation of water and inorganic nutrients

FIGURE 3-1 Ways in which pathogens reduce photosynthetic area and, thereby, photosynthesis in plants. (A) Spots on barley leaves caused by the fungus *Rhynchosporium sp*. (B) Nearly complete destruction of pumpkin leaves infected heavily with the downy mildew oomycete *Pseudoperonospora cubensis*. (C) Countless tiny lesions on stems and leaves of wheat plant infected with the stem rust fungus *Puccinia graminis f.sp. tritici*. is. (D) Angular leaf spots on cucumber leaf caused by the bacterium *Pseudomonas lacrymans*. (E) Reduced chlorophyll in yellowish areas of virus-infected plants, such as cowpea infected with *cowpea chlorotic mottle virus* or (F) by stunting and yellowing of rice plants infected with the *rice tungro virus*. [Photographs courtesy of (A) Plant Pathology Department, University of Florida, (B) T. A. Zitter, Cornell University (C) I. Evans and (D) R. J. Howard, W.C.P.D., and (F) H. Hibino.]

through plants. Some pathogens affect the integrity or function of the roots, causing them to absorb less water; other pathogens, by growing in the xylem vessels or by other means, interfere with the translocation of water through the stem; and, in some diseases, pathogens interfere with the water economy of the plant by causing excessive transpiration through their effects on leaves and stomata.

Effect on Absorption of Water by Roots

Many pathogens, such as damping-off fungi (Fig. 3-2A), root-rotting fungi and bacteria (Figs. 3-2B–3-2D), most nematodes, and some viruses, cause an extensive destruction of the roots before any symptoms appear on the aboveground parts of the plant. Some bacteria and nematodes cause root galls or root knots (Figs. 3-2E and 3-2F), which interfere with the normal absorption of water and nutrients by the roots. Root injury affects the amount of functioning roots directly and decreases proportionately the amount of water absorbed by the roots. Some vascular parasites, along with their other effects, seem to inhibit root hair production, which reduces water absorption. These and other pathogens also alter the permeability of root cells, an effect that further interferes with the normal absorption of water by roots.

Effect on Translocation of Water through the Xylem

Fungal and bacterial pathogens that cause damping off, stem rots (Fig. 3-3A), and cankers (Fig. 3-3B) may reach the xylem vessels in the area of the infection and, if the affected plants are young, may cause their destruction and collapse. Cankers in older plants, particularly older trees (Fig. 3-3B), may cause some reduction in the translocation of water, but, generally, do not kill plants unless the cankers are big or numerous enough to encircle the plant. In vascular wilts, however (Figs. 3-3C–3-3F), reduction in water translocation may vary from little to complete. In many cases, affected vessels may be filled with the bodies of the pathogen (Figs. 3-4A–3-4D) and with substances secreted by the pathogen (Figs. 3-5D and 3-5E) or by the host (Fig. 3-5C) in response to the pathogen and may become clogged (Figs. 3-4A and 3-4C and 3-5C–3-5E). Whether destroyed or clogged, the affected vessels cease to function properly and allow little or no water to pass through them. Certain pathogens, such as the crown gall bacterium (*Agrobacterium tumefaciens*), the clubroot protozoon (*Plasmodiophora brassicae*), and the root-knot nematode (*Meloidogyne* sp.), induce gall formation (Figs. 3-2E and 3-2F) in the stem, roots, or both. The enlarged and proliferating cells near or around the xylem exert pressure on the xylem vessels, which may be crushed and dislocated, thereby becoming less efficient in transporting water.

The most typical and complete dysfunction of xylem in translocating water, however, is observed in the vascular wilts (Figs. 3-3 and 3-5) caused by the fungi *Ceratocystis*, *Ophiostoma*, *Fusarium*, and *Verticillium* and bacteria such as *Pseudomonas*, *Ralstonia*, and *Erwinia*. These pathogens invade the xylem of roots and stems and produce diseases primarily by interfering with the upward movement of water through the xylem. In many plants infected by these pathogens the water flow through the stem xylem is reduced to a mere 2 to 4% of that flowing through stems of healthy plants. In general, the rate of flow through infected stems seems to be inversely proportional to the number of vessels blocked by the pathogen and by the substances resulting from the infection. Evidently more than one factor is usually responsible for the vascular dysfunction in the wilt diseases. Although the pathogen is the single cause of the disease, some of the factors responsible for the disease syndrome originate directly from the pathogen, whereas others originate from the host in response to the pathogen. The pathogen can reduce the flow of water through its physical presence in the xylem as mycelium, spores, or bacterial cells (Figs. 3-4A–3-4C and 3-5B) and by the production of large molecules (polysaccharides) in the vessels (Figs. 3-5D and 3-5E). In most host–pathogen combinations, the destruction of xylem vessels by fungi (Fig. 3-3A) results in the collapse and death of the plant, as does the invasion of xylem vessels by fungi (Figs. 3-3C and 3-3D) or bacteria (Figs. 3-3E and 3-3F and 3-5A–3-5F). In host combinations with the fastidious bacterium *Xylella fastidiosa*, growth, multiplication, and spread of bacteria in xylem vessels are slower and, instead of causing wilting and rapid death of the plant, a scorching of the margins of the leaves (Fig. 3-4D) and several other symptoms occur, but rarely does the plant die quickly. In all cases, however, in infected hosts the flow of water is reduced through reduction in the size or collapse of vessels due to infection, development of tyloses (Figs. 3-5C and 3-5E) in the vessels, release of large molecule compounds in the vessels as a result of cell wall breakdown by pathogenic enzymes (Figs. 3-5D and 3-5E), and reduced water tension in the vessels due to pathogen-induced alterations in foliar transpiration.

Effect on Transpiration

In plant diseases in which the pathogen infects the leaves, transpiration is usually increased. This is the result of destruction of at least part of the protection

FIGURE 3-2 Examples of reduction of water absorption by plants. (A) Destruction of roots of young seedlings by the damping-off oomycete *Pythium sp*. (B) Roots and stems of pepper plants killed by *Phytophthora sp*. (C) Wheat roots at different stages of destruction by the take-all fungus *Gaeumannomyces tritici*. (D) Infection of crown and roots of corn plant with the fungus *Fusarium*. (E) Numerous galls caused by the bacterium *Agrobacterium tumefaciens* on roots of a cherry tree. (F) Root knot galls caused by the nematode *Meloidogyne sp*. on roots of a cantaloupe plant. [Photographs courtesy of (A) Plant Pathology Department, University of Florida, (B) K. Pernezny, University of Florida, (C) W. McFadden, W.C.P.D., (D) Plant Pathology Department, Iowa State University, (E) Oregon State University, and (F) B. D. Bruton, USDA, Lane, Oklahoma.]

FIGURE 3-3 Examples of reduction of upward translocation of water and mineral nutrients by (A) the stem of a cantaloupe plant infected with the fungus *Phomopsis* sp. (B) Canker on an almond tree caused by the fungus *Ceratocystis fagacearum*. (C) Vascular wilt of tomato caused by the fungus *Fusarium*. (D) Discolored vascular tissues of a tomato stem infected with the same fungus. (E) Wilted tomato plants infected with the vascular bacterium *Ralstonia solanacearum*. (F) Discolored vascular tissues of a tomato plant infected with the same bacterium. [Photographs courtesy of (A) B. D. Bruton, USDA, Lane, Oklahoma, (B) B. Teviotdale, Kearney Agricultural Center, Parlier, California, (C,E, and F) Department of Plant Pathology, University Florida, and (D) L. McDonald, W.C.P.D.]

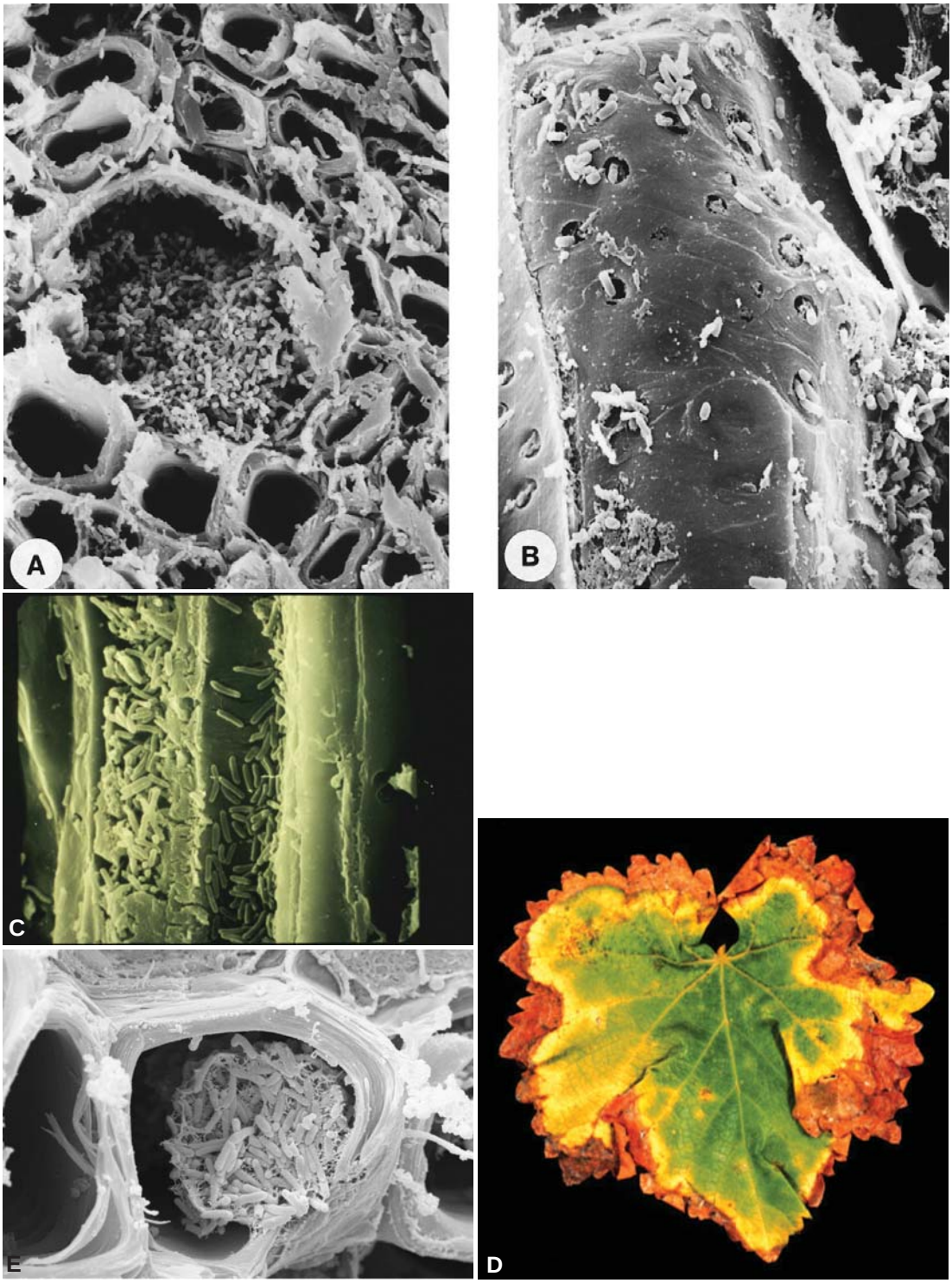

FIGURE 3-4 (A) *Pseudomonas* bacteria clogging a xylem vessel of a young plant shoot. (B) Bacteria moving from one vessel to another and to adjacent parenchyma cells through xylem pits. (C) Bacteria of the xylem-inhabiting *Xylella fastidiosa* in a vessel of a grape plant. (D) Marginal scorching of a grape leaf from a plant infected with *X. fastidiosa*, the cause of Pierce's disease of grape. (E) *Xylella* bacteria in a cross section of a xylem vessel of an infected grape leaf. [Photographs courtesy of (A and B) E. L. Mansvelt, I. M. M. Roos, and M. J. Hattingh (1500×), (C) D. Cooke, provided by E. Hellman, Texas A&M University, (D) E. Hellman, and (E) E. Alves, Federal University of Lavras, Brazil.]

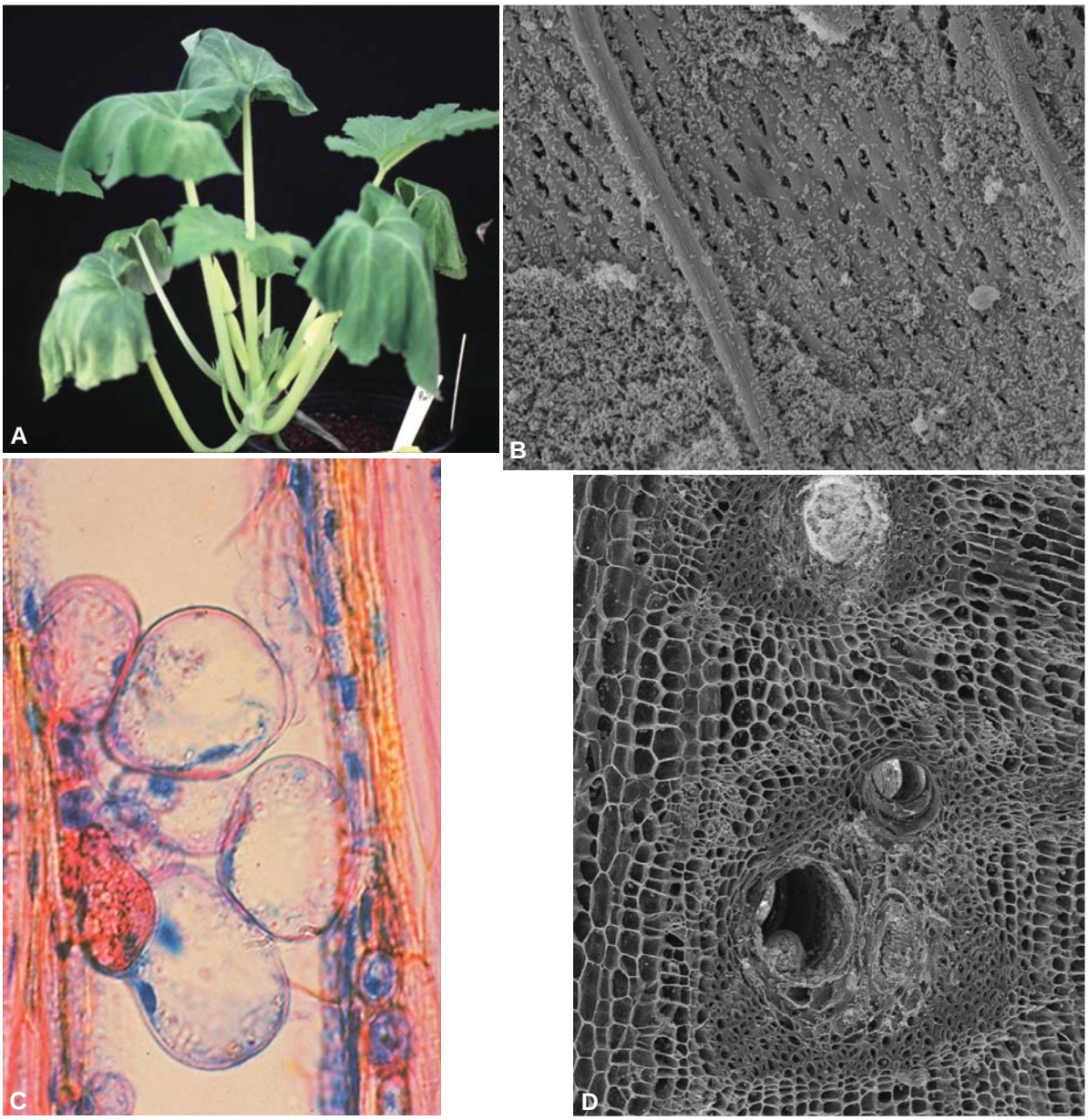

FIGURE 3-5 (A) Young squash plant showing early symptoms of vascular wilt caused by the bacterium *Erwinia tracheiphila*. (B) *E. tracheiphila* bacteria lining up the inside wall of a xylem vessel. (C) Tyloses in a xylem vessel. (D) Tyloses and gummy polysaccharides partially or totally clogging up xylem vessels of a squash plant. (E) Several xylem vessels totally clogged with gummy polysaccharides. (F) Cantaloupes in a field where the plants had been killed by the bacterium *E. tracheiphila*. [Photographs courtesy of (A,B,D,E, and F) B. D. Bruton, USDA, Lane, Oklahoma, and (C) D. M. Elgersma.]

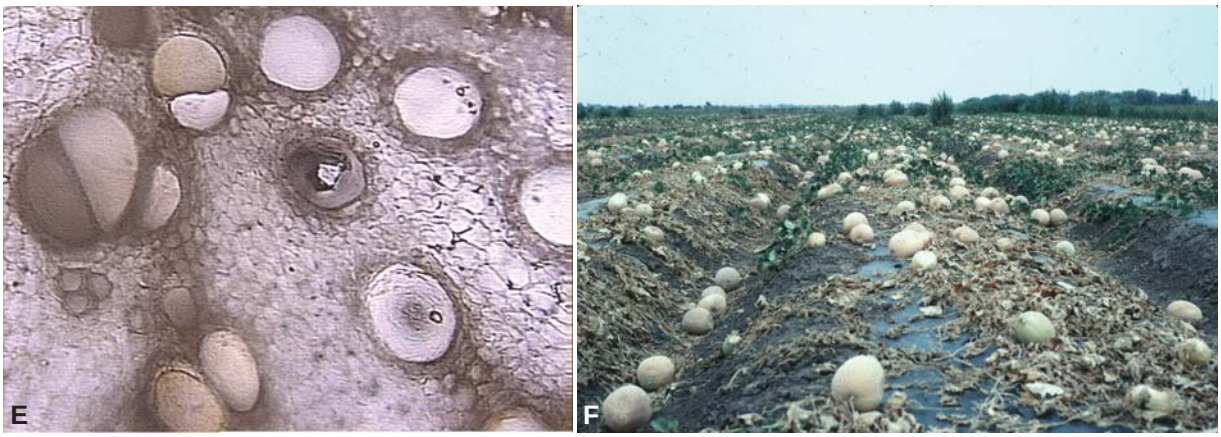

FIGURE 3-5 (*Continued*)

afforded the leaf by the cuticle, an increase in the permeability of leaf cells, and the dysfunction of stomata. In diseases such as rusts, in which numerous pustules form and break up the epidermis (Figs. 3-6A and 3-6B), in most leaf spots (Fig. 3-6E), in which the cuticle, epidermis, and all the other tissues, including xylem, may be destroyed in the infected areas, in the powdery mildews, in which a large proportion of the epidermal cells are invaded by the fungus (Fig. 3-6C), and in apple scab (Fig. 3-6D), in which the fungus grows between the cuticle and the epidermis—in all these examples, the destruction of a considerable portion of the cuticle and epidermis results in an uncontrolled loss of water from the affected areas. If water absorption and translocation cannot keep up with the excessive loss of water, loss of turgor and wilting of leaves follow. The suction forces of excessively transpiring leaves are increased abnormally and may lead to collapse or dysfunction of underlying vessels through the production of tyloses and gums.

Interference with Translocation of Organic Nutrients through the Phloem

Organic nutrients produced in leaf cells through photosynthesis move through plasmodesmata into adjoining phloem elements. From there they move down the phloem sieve tubes (Fig. 3-7) and eventually, again through plasmodesmata, into the protoplasm of living nonphotosynthetic cells, where they are utilized, or into storage organs, where they are stored. Thus, in both cases, the nutrients are removed from "circulation." Plant pathogens may interfere with the movement of organic nutrients from the leaf cells to the phloem, with their translocation through the phloem elements, or, possibly, with their movement from the phloem into the cells that will utilize them.

Obligate fungal parasites, such as rust and mildew fungi, cause an accumulation of photosynthetic products, as well as inorganic nutrients, in the areas invaded by the pathogen. In these diseases, the infected areas are characterized by reduced photosynthesis and increased respiration. However, the synthesis of starch and other compounds, as well as dry weight, is increased temporarily in the infected areas, indicating translocation of organic nutrients from uninfected areas of the leaves or from healthy leaves toward the infected areas.

In stem diseases of woody plants in which cankers develop (Figs. 3-8A–3-8C), the pathogen attacks and remains confined to the bark for a considerable time. During that time the pathogen attacks and may destroy the phloem elements in that area, thereby interfering with the downward translocation of nutrients. In diseases caused by phytoplasmas, as well as in diseases caused by phloem-limited fastidious bacteria, bacteria exist and reproduce in the phloem sieve tubes (Fig. 3-8D), thereby interfering with the downward translocation of nutrients. In several plants propagated by grafting a variety scion onto a rootstock, infection of the combination with a virus (e.g., infection of an apple or stone-fruit rootstock with *tomato ringspot virus*) leads to formation of a necrotic plate at the points of contact of the hypersensitive scion variety with the rootstock (Fig. 3-8E), which leads to the death of the scion. However, infection of a pear scion grafted on an oriental rootstock with the pear decline phytoplasma, or of a citrus variety propagated on sour rootstock with the citrus tristeza virus, results, in both cases, in the necrosis of a few layers of cells of each rootstock in contact with the tolerant variety. In these cases, the rootstock is the component of the scion/stock combination that is

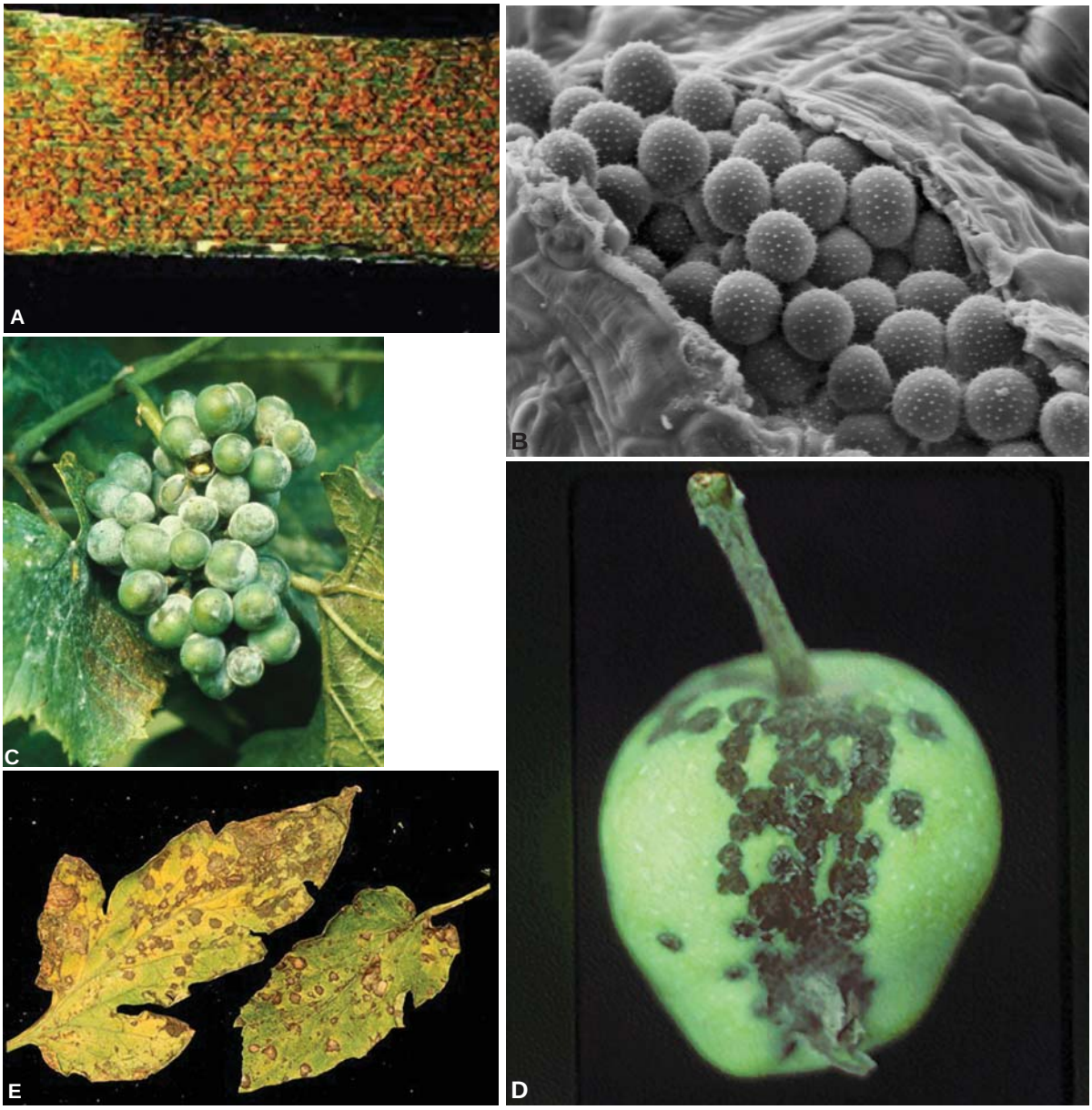

FIGURE 3-6 Ways by which pathogens cause increased transpiration in infected plants. (A) The wheat leaf rust pathogen *Puccinia recondita* produces innumerable lesions (uredia) on wheat leaves and causes millions of breaks in the leaf epidermis through which transpiration goes on uncontrollably. (B) Uredospores breaking the epidermis and emerging from the surface of an infected leaf. (C) Grape berries infected with the powdery mildew fungus *Uncinula necator*, the mycelium of which penetrates and forms haustoria in almost every epidermal cell. (D) The apple scab fungus *Venturia inaequalis* grows between the cuticle and the epidermis, causing the cuticle to break in numerous places, allowing transpiration to occur. (E) Tomato leaves with numerous lesions caused by the fungus *Septoria sp*. and through which excessive transpiration occurs. [Photographs courtesy of (A and E) W.C.P.D., (B) E. A. Richardson and C. W. Mims, University of Georgia, (C) J. Travis and J. Rytter, Plant Pathology Department, Pennsylvania State University, and (D) K. Mohan, University of Idaho.]

hypersensitive to and becomes killed by the appropriate pathogen.

In some virus diseases, particularly the leaf-curling type and some yellows diseases, starch accumulation in the leaves is mainly the result of degeneration (necrosis) of the phloem of infected plants (Fig. 3-8F), which is one of the first symptoms. It is also possible, however, at least in some virus diseases, that the interference with translocation of starch stems from inhibition by the virus of the enzymes that break down starch into smaller, translocatable molecules. This is suggested by the observation that in some mosaic diseases, in which there is no phloem necrosis, infected, discolored areas of leaves contain less starch than "healthy," greener areas at the end of the day, a period favorable for photosynthesis, but the same leaf areas contain more starch than the "healthy" areas after a period in the dark, which favors starch hydrolysis and translocation. This suggests not only that virus-infected areas synthesize less starch than healthy ones, but also that starch is not degraded and translocated easily from virus-infected areas, although no damage to the phloem is present.

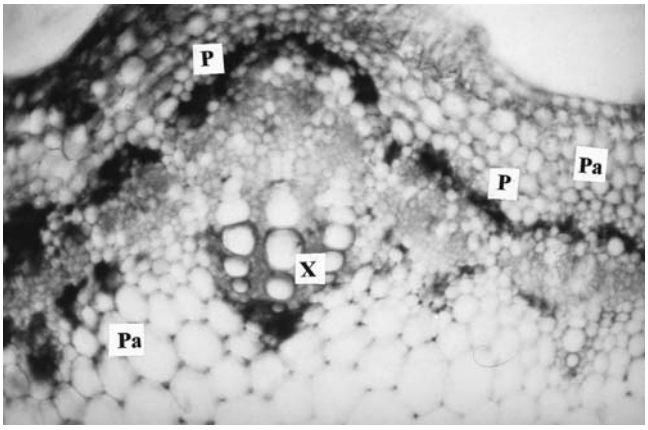

FIGURE 3-7 Necrosis of the phloem (P) in stems or petioles of plants is a common effect of viruses, such as the *tobacco ringspot virus*, on cowpea plants. As a result, roots starve and the plant declines (100×). Pa, parenchyma cells; X, xylem vessels.

EFFECT OF PATHOGENS ON HOST PLANT RESPIRATION

Respiration is the process by which cells, through the enzymatically controlled oxidation (burning) of the

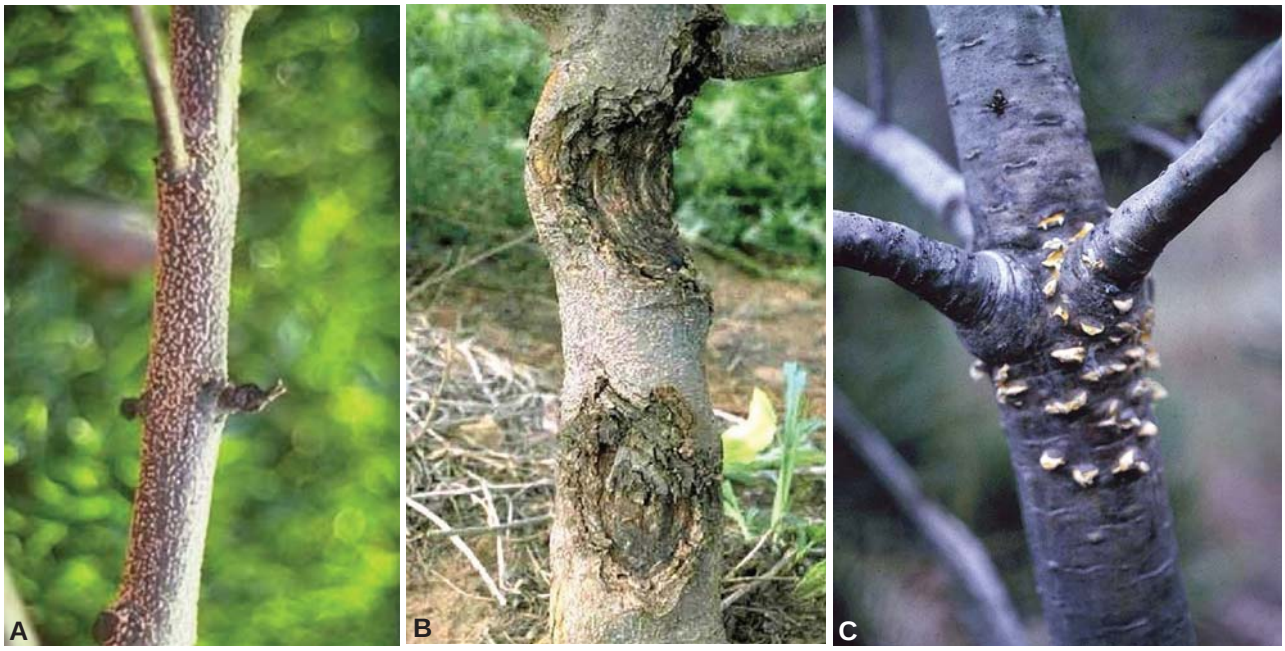

FIGURE 3-8 Examples of diseases in which the pathogen interferes with the downward translocation of organic nutrients. (A) Young canker caused by the fungus *Nectria* in which the bark of the branch has been invaded and killed by the fungus. (B) Two advanced *Nectria* cankers in which both the phloem and a great deal of the xylem have been killed by the fungus. (C) Blister canker on a pine tree in which the bark and phloem have been killed by the fungus *Cronartium ribicola*. (D) Phytoplasmas filling a phloem sieve element block the downward translocation of photosynthates. (E) The graft union of a pear grafted on oriental pear rootstocks, which results in the death of pear phloem. (F) Potato tuber showing vein necrosis caused by the *potato leaf roll virus*. [Photographs courtesy of (A) USDA Forest Service, (B) A. Jones, Plant Pathology Department, Michigan State University, (C) Oregon State University, and (F) Cornell University.

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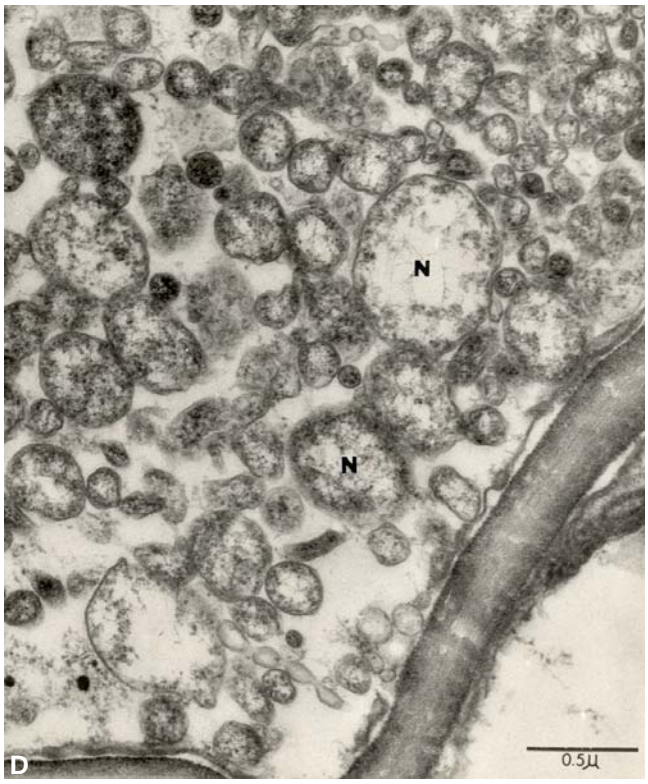

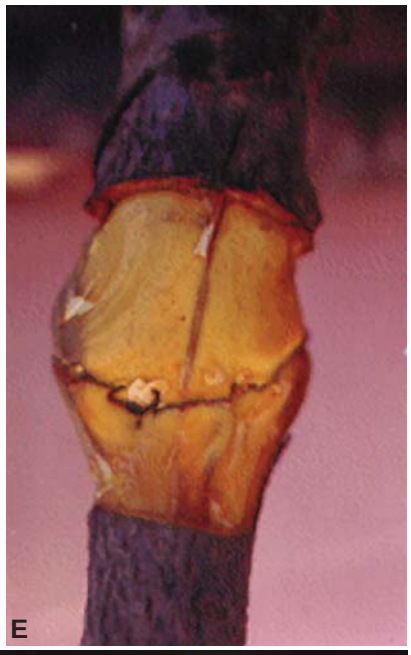

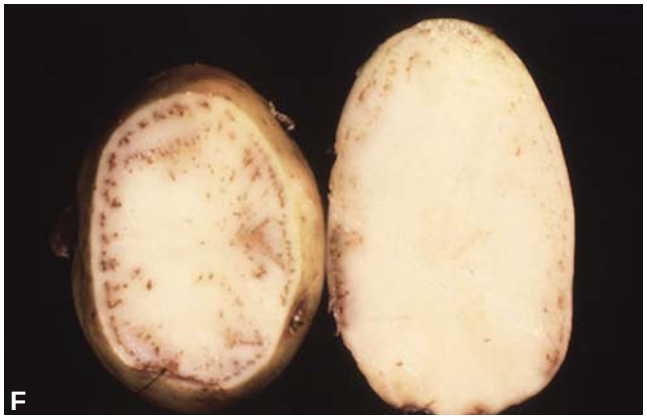

FIGURE 3-8 (*Continued*)

energy-rich carbohydrates and fatty acids, liberate energy in a form that can be utilized for the performance of various cellular processes. Plant cells carry out respiration in, basically, two steps. The first step involves the degradation of glucose to pyruvate and is carried out, either in the presence or in the absence of oxygen, by enzymes found in the ground cytoplasm of the cells. The production of pyruvate from glucose follows either the **glycolytic pathway**, otherwise known as **glycolysis**, or, to a lesser extent, the **pentose pathway**. The second step, regardless of the pathway, involves the degradation of pyruvate, however produced, to CO_2 and water. This is accomplished by a series of reactions known as the **Krebs cycle**, which is accompanied by the so-called **terminal oxidation** and is carried out in the mitochondria only in the presence of oxygen. Under normal (aerobic) conditions, i.e., in the presence of oxygen, both steps are carried out, and one molecule of glucose yields, as final products, six molecules of CO_2 and six molecules of water,

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$$

with a concomitant release of energy (678,000 calories). Some of the energy is lost, but almost half is converted to 20–30 reusable high-energy bonds of adenosine triphosphate (ATP). The first step of respiration contributes two ATP molecules per mole of glucose, and the second step contributes the rest. Under anaerobic conditions, however (i.e., in the absence of oxygen), pyruvate cannot be oxidized; instead it undergoes **fermentation** and yields lactic acid or alcohol. Because the main process of energy generation is cut off, for the cell to secure the necessary energy a much greater rate

of glucose utilization by glycolysis is required in the absence of oxygen than is in its presence.

The energy-storing bonds of ATP are formed by the attachment of a phosphate (PO_4) group to adenosine diphosphate (ADP) at the expense of energy released from the oxidation of sugars. The coupling of the oxidation of glucose with the addition of phosphate to ADP to produce ATP is called **oxidative phosphorylation**. Any cell activity that requires energy utilizes the energy stored in ATP by simultaneously breaking down ATP to ADP and inorganic phosphate. The presence of ADP and phosphate in the cell, in turn, stimulates the rate of respiration. If, however, ATP is not utilized sufficiently by the cell for some reason, there is little or no regeneration of ADP and respiration is slowed down. The amount of ADP (and phosphate) in the cell is determined, therefore, by the rate of energy utilization; this rate, in turn, determines the rate of respiration in plant tissues.

The energy produced through respiration is utilized by the plant for all types of cellular work, such as accumulation and mobilization of compounds, synthesis of proteins, activation of enzymes, cell growth and division, defense reactions, and a host of other processes. The complexity of respiration, the number of enzymes involved in respiration, its occurrence in every single cell, and its far-reaching effects on the functions and existence of the cell make it easy to understand why the respiration of plant tissues is one of the first functions to be affected when plants are infected by pathogens.

Respiration of Diseased Plants

When plants are infected by pathogens, the rate of respiration generally increases. This means that affected tissues use up their reserve carbohydrates faster than healthy tissues would. The increased rate of respiration appears shortly after infection — certainly by the time of appearance of visible symptoms — and continues to rise during the multiplication and sporulation of the pathogen. After that, respiration declines to normal levels or to levels even lower than those of healthy plants. Respiration increases more rapidly in infections of resistant varieties, in which large amounts of energy are needed and used for rapid production or mobilization of the defense mechanisms of the cells. In resistant varieties, however, respiration also declines quickly after it reaches its maximum. In susceptible varieties, in which no defense mechanisms can be mobilized quickly against a particular pathogen, respiration increases slowly after inoculation, but continues to rise and remains at a high level for much longer periods.

Several changes in the metabolism of the diseased plant accompany the increase in respiration after infection. Thus, the activity or concentration of several enzymes of the respiratory pathways seems to be increased. The accumulation and oxidation of phenolic compounds, many of which are associated with defense mechanisms in plants, are also greater during increased respiration. Increased respiration in diseased plants is also accompanied by an increased activation of the pentose pathway, which is the main source of phenolic compounds. Increased respiration is sometimes accompanied by considerably more fermentation than that observed in healthy plants, probably as a result of an accelerated need for energy in the diseased plant under conditions in which normal aerobic respiration cannot provide sufficient energy.

The increased respiration in diseased plants is apparently brought about, at least in part, by the uncoupling of oxidative phosphorylation. In that case, no utilizable energy (ATP) is produced through normal respiration, despite the use of existing ATP and the accumulation of ADP, which stimulates respiration. The energy required by the cell for its vital processes is then produced through other less efficient ways, including the pentose pathway and fermentation.

The increased respiration of diseased plants can also be explained as the result of increased metabolism. In many plant diseases, growth is at first stimulated, protoplasmic streaming increases, and materials are synthesized, translocated, and accumulated in the diseased area. The energy required for these activities derives from ATP produced through respiration. The more ATP is utilized, the more ADP is produced and further stimulates respiration. It is also possible that the plant, because of the infection, utilizes ATP energy less efficiently than a healthy plant. Because of the waste of part of the energy, an increase in respiration is induced, and the resulting greater amount of energy enables the plant cells to utilize sufficient energy to carry out their accelerated processes.

Although oxidation of glucose via the glycolytic pathway is by far the most common way through which plant cells obtain their energy, part of the energy is produced via the pentose pathway. The latter seems to be an alternate pathway of energy production to which plants resort under conditions of stress. Thus, the pentose pathway tends to replace the glycolytic pathway as the plants grow older and differentiate and it tends to increase on treatment of the plants with hormones, toxins, wounding, starvation, and so on. Infection of plants with pathogens also tends, in general, to activate the pentose pathway over the level at which it operates in the healthy plant. Because the pentose pathway is not linked directly to ATP production, the increased

respiration through this pathway fails to produce as much utilizable energy as the glycolytic pathway and is, therefore, a less efficient source of energy for the functions of the diseased plant. However, the pentose pathway is the main source of phenolic compounds, which play important roles in the defense mechanisms of the plant against infection.

EFFECT OF PATHOGENS ON PERMEABILITY OF CELL MEMBRANES

Cell membranes consist of a double layer of lipid molecules in which many kinds of protein molecules are embedded, parts of which usually protrude on one or both sides of the lipid bilayer (Fig. 5-2). Membranes function as permeability barriers that allow passage into a cell only of substances the cell needs and inhibit passage out of the cell of substances needed by the cell. The lipid bilayer is impermeable to most biological molecules. Small water-soluble molecules such as ions (charged atoms or electrolytes), sugars, and amino acids flow through or are pumped through special membrane channels made of proteins. In plant cells, because of the cell wall, only small molecules reach the cell membrane. In animal cells and in artificially prepared plant protoplasts, however, large molecules or particles may also reach the cell membrane and enter the cell by endocytosis, in which a patch of the membrane surrounds and forms a vesicle around the material to be taken in, brings it in, and releases it inside the cell. Disruption or disturbance of the cell membrane by chemical or physical factors alters (usually increases) the permeability of the membrane with a subsequent uncontrollable loss of useful substances, as well as the inability to inhibit the inflow of undesirable substances or excessive amounts of any substances.

Changes in cell membrane permeability are often the first detectable responses of cells to infection by pathogens, to most host-specific and several nonspecific toxins, to certain pathogen enzymes, and to certain toxic chemicals, such as air pollutants. The most commonly observed effect of changes in cell membrane permeability is the loss of **electrolytes**, i.e., of small water-soluble ions and molecules from the cell. Electrolyte leakage occurs much sooner and at a greater rate when the host–pathogen interaction is incompatible, and the host remains more resistant than when the host is susceptible and develops extensive symptoms (Fig. 3-9). It is not certain, however, whether the cell membrane is the initial target of pathogen toxins and enzymes and whether the accompanying loss of electrolytes is the initial effect of changes in cell membrane permeability

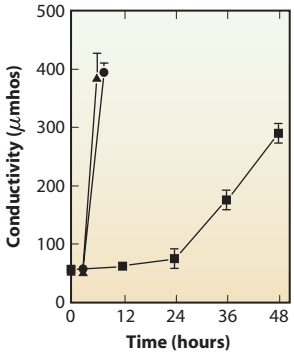

FIGURE 3-9 Levels of conductivity measuring the leakage of electrolytes released from leaves of pepper plants inoculated with three races of the bacterium *Xanthomonas campestris* pv. *vesicatoria*. (■) Release of electrolytes occurred later and at a slower rate when leaves were inoculated with a virulent race of the bacterium. (●,▲) Disruption of membranes and electrolyte leakage occurred much earlier, and at a much greater rate, when leaves were inoculated with two bacterial races carrying avirulence genes that triggered the hypersensitive response in plants carrying the corresponding resistance genes. [From Minsavage *et al.* (1990), *Mol. Plant-Microbe Interact.* 3, 41–47.]

or whether the pathogen products actually affect other organelles or reactions in the cell, in which case cell permeability changes and loss of electrolytes are secondary effects of the initial events. If pathogens do, indeed, affect cell membrane permeability directly, it is likely that they bring this about by stimulating certain membrane-bound enzymes, such as ATPase, which are involved in the pumping of H^+ in and K^+ out through the cell membrane, by interfering with processes required for the maintenance and repair of the fluid film making up the membrane, or by degrading the lipid or protein components of the membrane by pathogen-produced enzymes.

EFFECT OF PATHOGENS ON TRANSCRIPTION AND TRANSLATION

Transcription of cellular DNA into messenger RNA and translation of messenger RNA to produce proteins are two of the most basic, general, and precisely controlled processes in the biology of any normal cell (Fig. 3-10). The part(s) of the genome involved and the level and timing of transcription and translation vary with the stage of development and the requirements of each cell. Nevertheless, disturbance of any one of these processes, by pathogens or environmental factors, may cause drastic, unfavorable changes in the structure and function of the affected cells by its effect on the expression of genes.

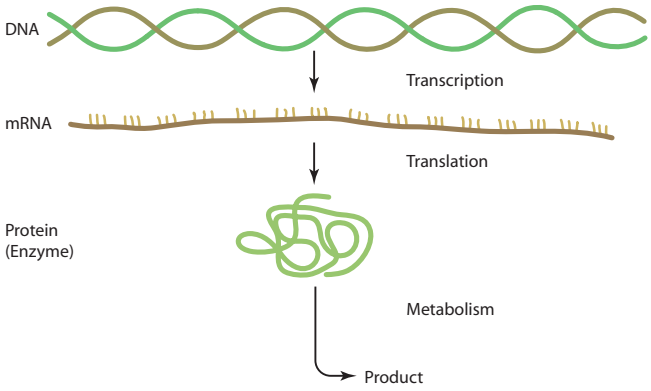

FIGURE 3-10 Transcription and translation processes.

Effect on Transcription

Several pathogens, particularly viruses and fungal obligate parasites, such as rusts and powdery mildews, affect the transcription process in infected cells. In some cases, pathogens affect transcription by changing the composition, structure, or function of the chromatin associated with the cell DNA. In some diseases, especially those caused by viruses, the pathogen, through its own enzyme or by modifying the host enzyme (RNA polymerase) that makes RNA, utilizes the host cell nucleotides and machinery to make its own (rather than host) RNA. In several diseases, the activity of ribonucleases (enzymes that break down RNA) is increased, perhaps by formation in infected plants of new kinds of ribonucleases not known to be produced in healthy plants. Finally, in several diseases, infected plants, particularly resistant ones, seem to contain higher levels of RNA than healthy plants, especially in the early stages of infection. It is generally believed that greater RNA levels and, therefore, increased transcription in cells indicate an increased synthesis of substances involved in the defense mechanisms of plant cells.

Effect on Translation

Infected plant tissues often have increased activity in several enzymes, particularly those associated with the generation of energy (respiration) or with the production or oxidation of various phenolic compounds, some of which may be involved in (defense) reactions to infection. Although a certain amount of some of these enzymes (proteins) may be present in the cell at the time of infection, several are produced *de novo*, necessitating increased levels of transcription and translation activity. Increases in protein synthesis in infected tissues have been observed primarily in hosts resistant to the pathogen and reach their highest levels in the early stages of infection, i.e., in the first few minutes and up to 2–20 hours after inoculation. If resistant tissues are treated before or during infection with inhibitors of protein synthesis, their resistance to the pathogen is reduced. These observations suggest that much of the increased protein synthesis in plants attacked by pathogens reflects the increased production of enzymes and other proteins involved in the defense reactions of plants.

EFFECT OF PATHOGENS ON PLANT GROWTH

It is easily understood and expected that pathogens that destroy part of the photosynthetic area of plants and cause significantly reduced photosynthetic output often result in smaller growth of these plants and smaller yields. Similarly, pathogens that destroy part of the roots of a plant or clog their xylem or phloem elements, thereby severely interfering with the translocation of water and of inorganic or organic nutrients in these plants, often cause a reduction in size and yields by these plants and, sometimes, their death. In many plant diseases, however, infected tissues or entire plants increase or reduce abnormally in size without a clear-cut explanation of how these changes are brought about. It is apparent that growth regulators affecting plant cell division and enlargement are involved, but very little is known about the specific compounds and mechanisms involved or the genes that control these events.

Some of the most common diseases in which pathogens cause obvious abnormal growth of their hosts' organs and tissues include clubroot of crucifers caused by the plasmodiophoromycete *Plasmodiophora brassicae*; alfalfa wart caused by the fungus *Physoderma alfalfae*, potato wart caused by the fungus *Spongospora subterranea*; peach leaf curl (Fig. 3-11A) and plum pockets (Fig. 3-11B) caused by the fungus *Taphrina sp.*, black knot canker of cherry caused by *Dibotryon morbosum* (Fig. 11-67A), Sphaeropsis gall of stone fruits caused by *Sphaeropsis sp.*; corn smut caused by *Ustilago maydis* (Figs. 5-16C and 11-144A–11-144C), dwarf bunt of wheat caused by *Tilletia contraversa* (Fig. 11-148), leaf gall of azalea caused by *Exobacidium azaleae* (Fig. 3-16A), and several rusts of pine trees caused by *Cronartium sp.* (Figs. 5-16D and 11-143). Some bacterial pathogens also cause abnormal growths such as crown gall (Fig. 3-11E) of many hosts and hairy root of apple caused by *Agrobacterium tumefaciens and A. rhizogenes*, respectively, olive knot and oleander gall caused by *Pseudomonas savastanoi*, and leafy gall of several hosts caused by *Rhodococcus sp.* (Fig. 5-17D).


A


B


C


D


E


F

Some characteristic effects on plant growth are caused by the phloem inhabiting phytoplasmas. Some phytoplasma-infected plants produce shoots that are yellowish, short, and bushy and are known as witches' brooms. Some phytoplasmas may cause stunting of their host and induce flower petals to become green as if they were leaves (known as phyllody). Nematodes are responsible for the very common root knot (Fig. 3-11F) of most cultivated plants caused by *Meloidogyne sp*.

The most frequent and unusual effects on plant growth are those caused by viruses (and viroids). Many viruses cause stunting (Fig. 3-11D) or dwarfing of infected plants, whereas others cause rolling or curling of leaves, abnormally shaped fruit, etc. Some viruses cause abnormalities even in the same leaf (Fig. 3-11C) where part of the leaf is thinner than normal and the rest is thicker than normal. Some viruses cause plants to produce galls on their root, stems, or leaves. Some induce pitting on the roots or stems of infected plants (Fig. 14-42E). How the various viruses bring about these effects on their respective hosts is not known.

EFFECT OF PATHOGENS ON PLANT REPRODUCTION

Pathogens that attack various organs and tissues of plants weaken and often kill these organs or tissues, thereby weakening the plants. As a result, such plants remain smaller in size, may produce fewer flowers, and may set fewer fruit and seeds; the latter may be of inferior vigor and vitality and, therefore, if planted, they may produce fewer and weaker new plants. In addition to these indirect effects of pathogens on plant reproduction, many pathogens have a direct adverse effect on plant reproduction because they attack and kill the flowers, fruit, or seed directly, or interfere and inhibit their production, or the pathogens interfere directly or indirectly with the propagation of their host plant.

One of the most common ways by which pathogens interfere with the reproduction of their host is by infecting and killing the flowers of the host, as happens, for example, with the brown rot of stone fruits caused by the fungus *Monilinia sp*. (Figs. 3-12A and 3-12B), the bacterial canker and gummosis of stone fruit trees caused by *Pseudomonas syringae*, and the fireblight disease of pears and apples caused by the bacterium *Erwinia amylovora*. In some diseases, e.g., in the postbloom fruit drop of citrus, the fruit, soon after set, drops prematurely as a result of infection by the anthracnose fungus *Colletotrichum acutatum*. Similarly, plums drop prematurely from trees infected with the *plum pox virus*. In several plant diseases, especially in grain crops, the pathogen interferes directly with the reproduction of the plant host by killing the embryo, that would have produced the seed, and replacing the contents of the seed with its own fruiting structure or its own spores. Examples of such diseases are ergot of grains (Fig. 3-12C), caused by the fungus *Claviceps purpurea;* corn smut (Fig. 3-12D); and the covered (Fig. 3-12E) and loose smuts of the various cereals caused by *Tilletia and Ustilago sp*., respectively. Finally, in some diseases caused by viruses, phytoplasmas, or phloem-limited bacteria, no flowers are produced or those produced are sterile, and therefore few or no fruit and seed are produced.

FIGURE 3-11 Effect of pathogens on plant growth. (A) Leaf curling and (B) fruit enlargement by the leaf curl fungus *Taphrina deformans* on peach and plum, respectively. (C) Leaf malformations caused by the *common bean mosaic virus* on bean and (D) a healthy and a plant showing stunting caused by the *maize streak virus* on corn (D). (E) Galls along the root and stem of a euonymus plant caused by the crown gall bacterium *Agrobacterium tumefaciens* and (F) galls along the roots of a plant caused by the root knot nematode *Meloidogyne sp*. [Photographs courtesy of (A and B) Oregon State University, (C) R. Provvidenti, Cornell University, (D) D. Coyne, Intrn. Inst. Trop. Agric., (E) R. Forster, Univ. of Idaho and (F) W. Crow, University of Florida.]

FIGURE 3-12 Ways in which pathogens affect plant reproduction. (A) Close-up of a flower and (B) macroscopic view of an apricot tree, the flowers of which have been killed by the brown rot fungus *Monilinia fructicola*. (C) A mixture of barley kernels (whitish-yellow) and ergot sclerotia (the larger black bodies) produced by the ergot fungus *Claviceps purpurea* on the heads of grain crops in place of healthy kernels. (D) Ear of corn having some of the corn kernels replaced by galls containing spores of the fungus *Ustilago maydis*. (E) A mixture of intact healthy wheat kernels and somewhat darker, broken wheat kernels filled with spores of the common bunt (covered smut) fungus *Tilletia* sp. [Photographs courtesy of (A and B) I. MacSwain, Oregon State University, (C) G. Munkvold, Iowa State University, (D) T. Zitter, Cornell University, and (E) J. Riesselman, USDA, Montana State University.]

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Chapter four

GENETICS *OF* PLANT DISEASE

INTRODUCTION

The genetic information of all organisms, i.e., the information that determines what an organism can be and can do, is encoded in its deoxyribose nucleic acid (DNA). In RNA viruses, of course, it is encoded in their ribose nucleic acid (RNA). In all organisms, most DNA is present in the chromosome(s). In prokaryotes, such as bacteria and mollicutes, which lack an organized, membrane-bound nucleus, there is only one chromosome and it is present in the cytoplasm, whereas in eukaryotes, i.e., all other organisms except viruses, there

are several chromosomes and they are present in the nucleus. Many prokaryotes, however, and some of the lower eukaryotes also carry smaller circular molecules of DNA called **plasmids** in the cytoplasm. Plasmid DNA also carries genetic information but multiplies and moves independently of the chromosomal DNA. Furthermore, all cells of eukaryotic organisms carry DNA in their mitochondria. Plant cells, in addition to nuclear and mitochondrial DNA, also carry DNA in their chloroplasts (Fig. 4-1).

Genetic information in DNA is encoded in a linear fashion in the order of the four bases (A, adenine; C, cytosine; G, guanine; and T, thymine). Each triplet of adjacent bases codes for a particular amino acid. A **gene** is a stretch of a DNA molecule, usually of about 100 to 500 or more adjacent triplets, that codes for one protein molecule or, in a few cases, one RNA molecule (Fig. 4-2). In eukaryotes, the coding region of a gene is often interrupted by noncoding stretches of DNA called **introns** (Fig. 4-3). When a gene is active, i.e., is expressed, one of its DNA strands is used as a template and is transcribed into an RNA strand. Some genes code only for an RNA and that RNA is either a transfer RNA (tRNA) or a ribosomal RNA (rRNA). Most genes encode proteins, however, and the transcription product is a messenger RNA (mRNA). The mRNA then becomes attached to ribosomes, which, with the help of tRNAs, translate the base sequence of the mRNA strand into a specific sequence of amino acids that folds into a specific shape and forms a particular protein. Different genes code for different proteins. Some of the proteins are part of the structure of cell membranes, but most act as enzymes. Proteins give cells and organisms their characteristic properties, such as shape, size, and color; determine what kinds of chemical substances are produced by the cell; and regulate all activities of cells and organisms.

Of course, not all genes in a cell are expressed at all times, as different kinds of cells at different times have different functions and needs. Which genes are turned on, when they are turned on or off, and for how long they stay on are all regulated by additional stretches of DNA called **promoters, enhancers, silencers**, or **terminators**. These act as signals for genes to be expressed or to stop being expressed or they act as signals for the production of RNAs and proteins that themselves act as inducers, promoters, and enhancers of gene expression or as repressors and terminators of gene expression. In many cases of host–pathogen interaction, genes in the one organism are triggered to be expressed by a substance produced by the other organism. For example, genes for cell wall-degrading enzymes in the pathogen are apparently induced by the presence of monomers or oligomers of host cell wall macromolecules that are substrates for these enzymes. Also, genes for defense reactions in the host, e.g., the production of phytoalexins, apparently are triggered to expression by certain signal compounds activated by inducer molecules (elicitors) produced by the pathogen.

GENES AND DISEASE

When different plants, such as tomato, apple, or wheat, become diseased as a result of infection by a pathogen, the pathogen is generally different for each kind of host plant. Moreover, the pathogen is often specific for that particular host plant. Thus, the fungus *Fusarium oxysporum* f. sp. *lycopersici*, which causes tomato wilt, attacks only tomato and has absolutely no effect on

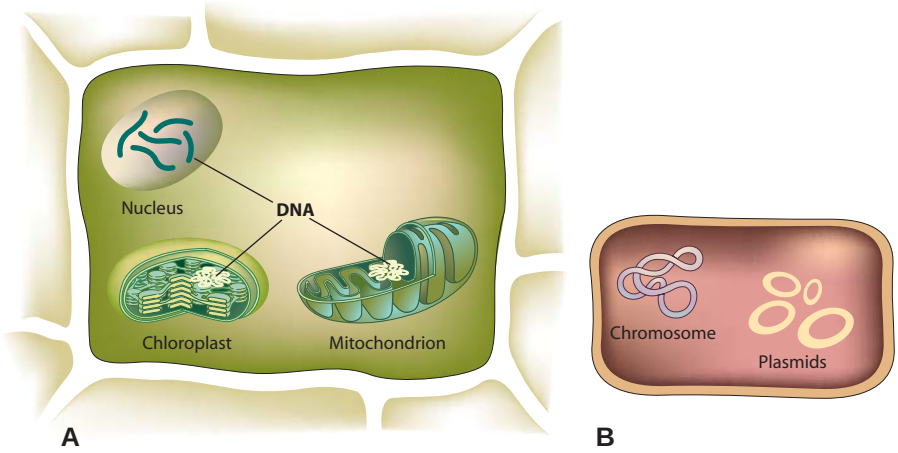

FIGURE 4-1 Location and arrangement of the genetic material in (A) eukaryotic (plant) cells and (B) prokaryotic (bacterial) cells.

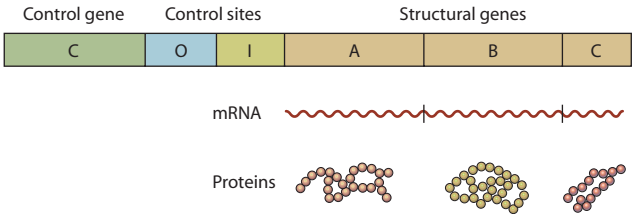

FIGURE 4-2 Gene structure, control, and expression in prokaryotes.

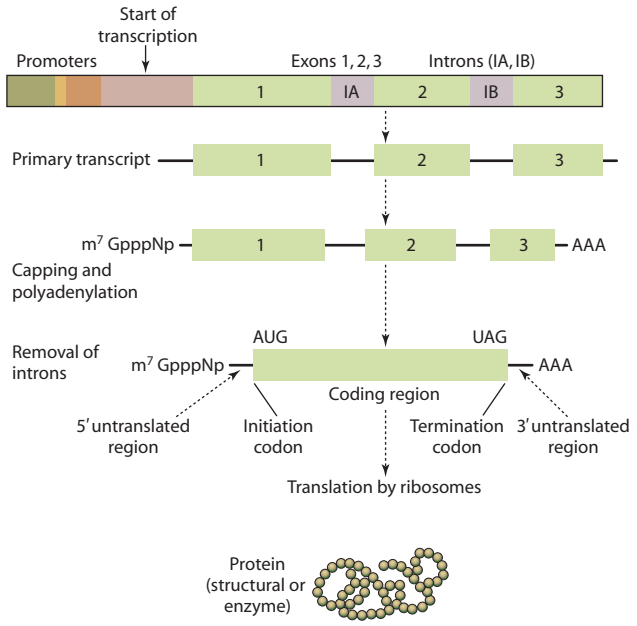

FIGURE 4-3 Gene structure, control, and expression in eukaryotes.

apple, wheat, or any other plant. Similarly, the fungus *Venturia inaequalis*, which causes apple scab, affects only apple, whereas the fungus *Puccinia graminis* f. sp. *tritici*, which causes stem rust of wheat, attacks only wheat. What makes possible the development of disease in a host is the presence in the pathogen of one or more genes for pathogenicity, for specificity, and for virulence against the particular host.

The gene(s) for virulence in a pathogen is usually specific for one or a few related kinds of plants that are hosts to the pathogen. Also, the genes and gene combinations that make a plant susceptible, i.e., a host to a particular pathogen, are present only in that one kind of plant and possibly a few related kinds of plants. All plants also have preformed and induced defenses that provide resistance against most pathogens. The specificity of microbial virulence genes that condition growth and disease on particular plants explains why a pathogen that is virulent on one kind of plant is not able to attack other kinds of plants and why a plant that is susceptible to one pathogen is not susceptible to all other pathogens of other host plants. This is known as nonhost resistance (Figs. 4-4 and 4-5).

Of course, a few pathogens are able to attack many kinds, sometimes hundreds, of host plants. Such pathogens tend to be necrotrophs and can attack so many hosts apparently because they either have many diverse genes for virulence or, more likely, because their genes of virulence somehow have much less plant specificity than those of the commonly more specialized pathogens. Each species of plant, however, seems to be susceptible to a fairly small number of different pathogens, usually less than a hundred for most plants.

Despite the many pathogens that can infect them, sometimes a few and many times countless numbers of individuals of a single plant species, such as corn, wheat, or soybean, survive in huge land expanses year after year. These plants survive either free of disease or with only minor symptoms, even though most of the other plants in the field have been killed (Fig. 4-5) and their pathogens are often widespread among the surviving plants. Why are all the plants not attacked by their pathogens, and why are those that are attacked not usually killed by the pathogens? The answer is complex, but basically it happens because plants, through evolution or through systematic breeding, have acquired, in addition to the genes that make them susceptible to a pathogen, one or usually numerous genes for resistance that protect the plants from infection or from severe disease. When a new gene for resistance to a pathogen

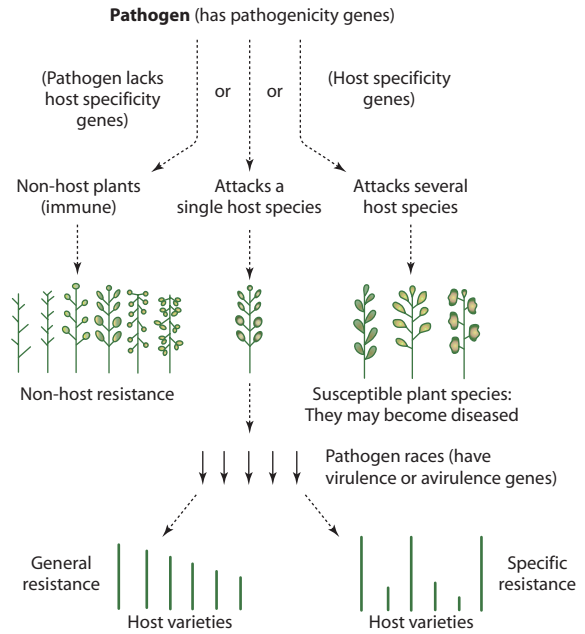

FIGURE 4-4 General interactions of a pathogen with its host and nonhost plants.

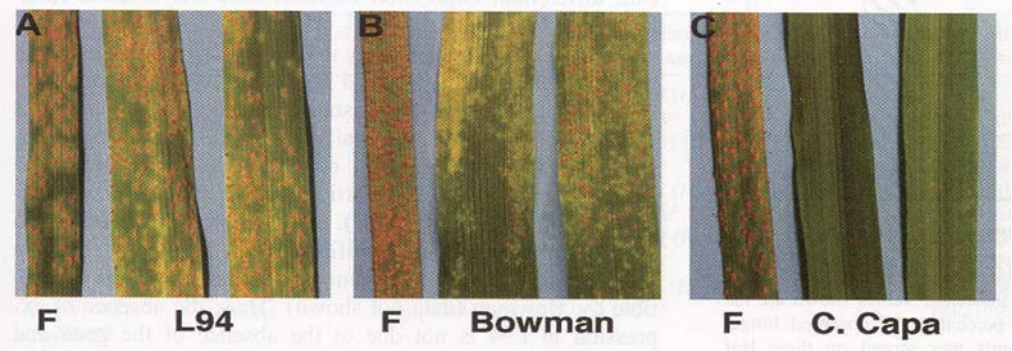

FIGURE 4-5 Infection types of two seedling leaves from each of three barley cultivars 10 days after artificial inoculation with the inappropriate wheat leaf rust fungus *Puccinia triticina*. Wheat cultivar F was used as the susceptible control. Only the cultivar C. Capa behaved as a nonhost. Infection of the others was bridged by a pathogen presumably but apparently not limited to wheat. [Photograph courtesy of Feuillet *et al.* (2003). *Mol. Plant-Microbe Interact.* **16**, 626–633.]

appears or is introduced into a plant, the plant becomes resistant to all or most of the previously existing individuals of the pathogen. Such pathogens contain one and usually more than one gene for virulence, but if they do not contain the additional new gene for virulence that is required to overcome the effect of the new resistance gene in the plant, they cannot infect the plant and the plant remains resistant. Thus, even one new gene for resistance to a pathogen can protect plants that have the gene from becoming infected by all or most preexisting races of the pathogen — at least for several months and possibly for several years.

It has been the experience of researchers with numerous host–pathogen combinations, however, that, after a new gene for resistance to a pathogen is introduced into a crop variety and that variety is planted in the fields, a new population (race) of the pathogen appears that contains a new gene for virulence that enables the pathogen to attack the crop plants containing the new gene for resistance. How did this new population of pathogens acquire the new gene for virulence? In most cases the new gene had already been present earlier at low levels, or by mutation, but only in a few pathogen individuals. New genes can arise randomly and suddenly *de novo* through mutations, or by rearrangement of the genetic material of the pathogens through the ever-ongoing events of genetic variability in organisms. Such pathogen individuals may have been but a tiny proportion of the total pathogen population and were undetected before plants with the new resistance gene were planted widely. After such plants were introduced, however, the new resistance gene excluded all other pathogen individuals except the few containing the new gene for virulence, which could attack these plants. Exclusion of the pathogens that lacked the new gene allowed the few that carried the gene to multiply and take over.

VARIABILITY IN ORGANISMS

One of the most dynamic and significant aspects of biology is that characteristics of individuals within a species are not "fixed," i.e., they are not identical but vary from one individual to another. As a matter of fact, all individuals produced as a result of a sexual process, such as the children of one family, are expected to be different from one another and from their parents in a number of characteristics, although they retain most similarities with them and belong to the same species. This is true oomycetes and of fungi produced from sexual spores such as oospores, ascospores, and basidiospores; of parasitic higher plants produced from seeds; and of nematodes produced from fertilized eggs, as well as of cultivated plants produced from seeds. Even bacteria have mechanisms for the transfer of genetic information. When individuals are produced asexually, the frequency and degree of variability among the progeny are reduced greatly, but even then certain individuals among the progeny will show different characteristics. Because of the astronomical number of individuals produced by microorganisms asexually, the total amount of variability produced by at least some microorganisms is probably as great and possibly greater than the total variability found in microorganisms reproducing sexually. This is the case in the overwhelmingly asexual reproduction of fungi by means of conidia, zoospores, sclerotia, and uredospores, and in bacteria, mollicutes, and viruses.

MECHANISMS OF VARIABILITY

In host plants and in pathogens, such as most fungi, parasitic higher plants, and nematodes, which can, and

usually do, reproduce by means of a sexual process, variation in the progeny is introduced primarily through segregation and recombination of genes during the meiotic division of the zygote. Bacteria too, and even viruses, exhibit variation that seems to be the result of a sexual process. In many fungi, heteroploidy and certain parasexual processes lead to variation. However, all plants and all pathogens, especially bacteria, viruses, and fungi, and probably mollicutes, can and do produce variants by means of mutations in the absence of any sexual process.

General Mechanisms of Variability

Two mechanisms of variability, namely mutation and recombination, occur in both plants and pathogens.

Mutation

A mutation is a more or less abrupt change in the genetic material of an organism, which is then transmitted in a hereditary fashion to the progeny. Mutations represent changes in the sequence of bases in the DNA either through substitution of one base for another or through addition or deletion of one or many base pairs. Additional changes may be brought about by amplification of particular segments of DNA to multiple copies; by insertion or excision of a transposable element, i.e., a movable DNA segment, into a coding or regulatory sequence of a gene; and by inversion of a DNA segment. On average, one mutation occurs for every million copies of a gene per generation. Since the average fungus genome consists of about 10,000 genes, one cell in a hundred could be a mutant or, stated differently, there are many mutants in every colony of a fungus or a bacterium, etc. Mutation at a locus that codes for an enzyme can result in an allele that produces an altered form of the enzyme, often called an allozyme. Mutations occur spontaneously in nature in all living organisms: those that produce only sexually or only asexually and those that reproduce both sexually and asexually. Mutations in single-celled organisms, such as bacteria, in fungi with a haploid mycelium, and in viruses, may be expressed immediately after their occurrence. Most mutations, however, are usually recessive; therefore, in diploid or dikaryotic organisms, mutations can remain unexpressed until they are brought together in a homozygous condition.

Mutations for virulence probably occur no more frequently than mutations for any other inherited characteristics, but given the great number of progeny produced by pathogens, it is probable that large numbers of mutants differing in virulence from their parent are produced in nature every year. In addition, considering that only a few genetically homogeneous varieties of each crop plant are planted continuously over enormous land expanses for a number of years, and considering the difficulties involved in shifting from one variety to another on short notice, the threat of new, more virulent, mutants appearing and attacking a previously resistant variety is a real one. Moreover, once a new factor for virulence appears in a mutant, this factor will take part in the sexual or parasexual processes of the pathogen and may produce recombinants possessing virulence quite different in degree or nature from that existing in the parental strains.

Because plants and pathogens contain genetic material (DNA) outside the cell nucleus in the form of organelle or plasmid DNA or even as double-stranded RNA, mutations in the extranuclear DNA are just as common as those in the nuclear DNA and affect whatever characteristics are controlled by the extranuclear DNA. Because the inheritance of characteristics controlled by extranuclear DNA (cytoplasmic inheritance) does not follow the Mendelian laws of genetics, mutations on that DNA are more difficult to detect and characterize. Through mutations in extrachromosomal DNA, many pathogens acquire (or lose) the ability to carry out a physiological process that they could not (or could) before. Cytoplasmic inheritance presumably occurs in all organisms except viruses and viroids, which lack cytoplasm. Three types of adaptations brought about by changes in the genetic material of the cytoplasm have been shown in pathogens: Pathogens may acquire the ability to tolerate previously toxic substances, to utilize new substances for growth, and to change their virulence toward host plants. Several characteristics of plants are also inherited through extranuclear DNA, including resistance or susceptibility to infection by certain pathogens.

Recombination

Recombination occurs primarily during the sexual reproduction of plants, fungi, and nematodes whenever two haploid (1N) nuclei, containing genetic material that may differ in many loci, unite to form a diploid (2N) nucleus, called a zygote. The zygote, sooner or later, divides meiotically and produces new haploid cells (gametes, spores, mycelium). Recombination of genetic factors (different genes or alleles of the same genes) occurs during the meiotic division of the zygote as a result of genetic crossovers in which parts of chromatids (and the genes they carry) of one chromosome of a pair are exchanged with parts of chromatids of the other chromosome of the pair. Recombination of the genes of two parental nuclei takes place in the zygote, and the

eventual haploid nuclei or gametes resulting after meiosis are different both from the gametes that produced the zygote and from one another. Over time, an organism may accumulate several alleles of a gene that code for slightly different forms of an enzyme, called isozymes. Such enzymes are controlled by genes at different loci and function under slightly different conditions of temperature, pH, etc. Recombination can also occur during mitotic cell division in the course of growth of an individual and it is thought to account for a significant amount of genetic exchange in fungi. In the fungi, haploid nuclei or gametes often divide mitotically to produce haploid mycelium and spores, which results in genetically different groups of relatively homogeneous individuals that may produce large populations asexually until the next sexual cycle.

Gene and Genotype Flow among Plant Pathogens

Gene flow is the process by which certain alleles (genes) move from one population to another geographically separated population. In plant pathology, gene flow is very important because it deals with the movement of virulent mutant alleles among different field populations. High gene flow in a pathogen increases the size of the population and of the geographical area in which its genetic material occurs. Therefore, pathogens that show a high level of gene flow generally have greater genetic diversity than pathogens that show a low level of gene flow. In pathogens reproducing only asexually, in which no recombination occurs, entire genotypes can be transferred from one population to another. This is known as genotype flow. Pathogens that produce hardy spores or other propagules, such as rust and powdery mildew fungi, that can spread over long distances, can distribute their genomes over large areas, sometimes encompassing entire continents. However, soil-borne fungi and nematodes move slowly and are present in small areas and their level of genetic flow is limited. With all types of pathogens, however, their gene flow can be affected significantly by human agricultural practices and by intercontinental travel and commerce. In general, pathogens with a high level of gene flow or genotype flow are much more effective and pose a greater threat to agriculture than pathogens with a low level of gene flow. Also, because asexual spores and propagules contain an already well-adapted and selected set of alleles, such propagules, through their genotype flow, pose a greater threat in enlarging the area of their adaptation than sexual propagules through their gene flow.

The frequency of alleles of importance in a population is affected by gene flow from other populations. Its magnitude depends on the number of incoming outside individuals into the population compared to the size of the population, as well as the number of different alleles brought into the population by outside individuals. Usually, allele frequencies in small populations adjacent to large ones are influenced strongly by gene flow than under any different conditions. Gene flow between distant populations is generally sporadic unless it is facilitated by intervening populations that act as stepping stones for the pathogen. The effect of gene flow is to reduce genetic differences between populations, thereby preventing or delaying the evolution of the populations in different geographical areas into separate species of the pathogen.

Population Genetics, Genetic Drift, and Selection

The size of a population affects the frequency of survival of mutants and thereby the diversity of genes in the population. Populations of most organisms in a geographic area may not be large enough to ensure that each variant will have progeny in the next generation so that random effects would occur during the transmission of genetic traits to new generations. This is known as random genetic drift. Because mutation rates are generally low (about one in a million), large populations are expected to have more mutants than small populations (a population of one million would have one mutant, another one of one billion would have 1,000 mutants). It is obvious that it is more likely that the one mutant of the smaller population will be lost than the 1,000 mutants of the larger population, i.e., in small populations, genetic drift results in a loss of alleles over time. In plant pathology, pathogens that exist in large populations have a greater potential for evolution than pathogens that exist in small populations. Large populations increase the probabilities that new mutants with greater fitness will emerge within a host, will be able to multiply in it, and will spread to a new host before the mutation is lost through genetic drift. Also, cultural practices, including chemical control, which regularly severely reduce pathogen populations in the field, are less diverse and much slower to adapt than populations that are allowed to maintain high populations year round.

Selection is a directional process by which the fittest variants in a particular environment increase their frequency in the population (positive selection), whereas less fit variants decrease their frequency (negative selection). As a result of selection in a population large enough for all variants to have progeny in the next generation, the frequency of a variant at equilibrium provides an estimate of the fitness of the variant. Selection results in a decrease in the diversity within a population, but it may cause an increase in the diversity between populations. Selection is affected by almost every factor

in the life cycle of a pathogen, whether related to the pathogen itself, to its host, its vector if any, and to the environment.

Life Cycles: Reproduction — Mating Systems — Outcrossing

Life cycles of various plant pathogens vary considerably, being most complex in some oomycetes and fungi. While life cycles are very simple, and basically asexual, in bacteria and in mitosporic fungi, in most fungi and in oomycetes they can involve a strictly haploid life cycle, a haploid–dikaryotic life cycle, a haploid–diploid one, a diploid one, or an asexual one. The kind of life cycle and the mating system followed affect the opportunities and limitations for genetic diversity (gene or genome diversity) and evolution of each particular pathogen. As a brief example we will mention the wheat stem rust fungus *Puccinia graminis* f. sp. *tritici*, which in the haploid state infects barberry while in the diploid state it infects wheat. Reproduction can be sexual, asexual, or both. The mating system is important only in relation to the sexual component of reproduction and can vary from inbreeding to outcrossing. In asexual pathogen populations, genotype diversity is more significant than gene diversity, whereas sexual pathogen populations show more gene diversity. Therefore, pathogens undergoing any type of recombination pose a greater threat than pathogens that undergo little or no recombination. The result of this is that the recombining pathogen population can put together new combinations of virulence genes or alleles as fast as breeders can put together genes for resistance and, therefore, pyramiding resistance genes in plants may not be as effective a strategy for as long as plant breeders hoped it would. Also, pathogens that outcross, through which more new genotypes are created, pose a greater threat to crops than inbreeding pathogens.

Pathogen Fitness

Fitness is the ability of a pathogen to survive and reproduce. The fitness of a pathogen or parasite can be quantified by measuring its reproductive rate, rate of multiplication, efficiency of infection, and amount of disease caused (aggressiveness). Fitness seems to be the driving force in the stability and evolution of a pathosystem in agriculture. In a freely mating system, excess virulence genes in a pathogen population constitute a genetic load or drag so that future selection favors genotypes free of excess genes. Even the presence of excess genes for virulence imposes a fitness penalty to the pathogen. Therefore, a mutation from avirulence to virulence occurs only if it is needed to overcome an R gene for resistance, i.e., only if it is absolutely necessary for the pathogen to survive. So, for a specific interaction between a pathogen with an avirulence gene and a host with a matching R gene for resistance, a mutation to virulence will occur because it increases the fitness of the pathogen to survive while the R gene is present. If, however, the mutation from avirulence to virulence gene carries a fitness penalty, the pathogen will suffer from reduced fitness on the host in the absence of the R gene. Many genes coding for fitness attributes or for virulence also encode the avirulence or host recognition function. Therefore, if loss of the function for avirulence is associated with a cost to fitness, represented by k, then the reduced fitness of the gene should appear on both host varieties, the one with and the one without the corresponding R gene resistance (1-k). It has been suggested that if this is true, then the greater the cost of fitness (the greater the value of k), the more durable the resistance of the variety is likely to be. Although some experimental data support this hypothesis, others are inconclusive.

Specialized Mechanisms of Variability in Pathogens

Certain mechanisms for generating variability appear to operate only in certain kinds of organisms or to operate in a rather different manner than those described as general mechanisms of variability. These specialized mechanisms of variability include heterokaryosis, heteroploidy, and parasexualism in fungi; conjugation, transformation, and transduction in bacteria; and genetic recombination in viruses.

Sexual-like Processes in Fungi

Heterokaryosis

Heterokaryosis is the condition in which, as a result of fertilization or anastomosis, cells of fungal hyphae or parts of hyphae contain two or more nuclei that are genetically different. For example, in Basidiomycetes, the dikaryotic state may differ drastically from the haploid mycelium and spores of the fungus. In *P. graminis tritici*, the fungus causing stem rust of wheat, the haploid basidiospores can infect barberry but not wheat, and the haploid mycelium can grow only in barberry; however, the dikaryotic aeciospores and uredospores can infect wheat but not barberry, and the dikaryotic mycelium can grow in both barberry and wheat. Heterokaryosis also occurs in other fungi, but its importance in plant disease development in nature is not known.

Parasexualism

Parasexualism is the process by which genetic recombinations can occur within fungal heterokaryons. This comes about by the occasional fusion of the two nuclei and formation of a diploid nucleus. During multiplication, crossing-over occurs in a few mitotic divisions and results in the appearance of genetic recombinants as the diploid nucleus progressively and rapidly loses individual chromosomes to revert to its haploid state. Considering that fungi exist and grow primarily as adjacent hyphae that may form heterokaryons as a result of anastomoses or fertilization, the frequency of parasexualism and therefore of genetic variability through parasexualism may equal or surpass that brought about by sexual reproduction.

Vegetative Incompatibility

In many fungi, vegetative hyphae of the same colony, or of two colonies of the same species, coming in contact with each other, often fuse, and the fusion is called hyphal anastomosis. If, however, hyphae coming in contact belong not to different strains of the fungus but of the same species, no fusion of hyphae takes place and the phenomenon is called vegetative incompatibility (or somatic or heterokaryon incompatibility). In only a few filamentous fungi, such as the species *Thanatephorus cucumeris*, the telomorph of *Rhizoctonia solani*, does fusion incompatibility occur between distantly related strains that appear to be different species, but when it does occur, it prevents both vegetative fusion and sexual fusion and, thereby, does not allow the exchange of genetic material. It has been suggested, therefore, that perhaps the different fusion incompatibility groups constitute different biological species still unrecognized within the broad species of *T. cucumeris*.

When hyphae from two colonies that belong to different postfusion incompatibility groups meet, the hyphae fuse, but subsequently the protoplasm in the two fused hyphal compartments and some adjacent ones is destroyed and a demarcation zone of sparse and sometimes dark mycelium is produced. Such postfusion incompatibility is the result of interaction between two alleles of the same vegetative compatibility (v-c) locus and is called allelic incompatibility. Vegetative incompatibility appears to be a defense mechanism that protects individuals from harmful nuclei, mitochondria, plasmids, and viruses that could reach them from other cells through anastomosis.

Heteroploidy

Heteroploidy is the existence of cells, tissues, or whole organisms with numbers of chromosomes per nucleus that are different from the normal 1N or 2N complement for the particular organism. Heteroploids may be haploids, diploids, triploids, or tetraploids or they may be aneuploids, i.e., have one, two, three, or more extra chromosomes or are missing one or more chromosomes from the normal euploid number (e.g., N + 1). Heteroploidy is often associated with cellular differentiation and represents a normal situation in the development of most eukaryotes. In several studies, spores of the same fungus were found to contain nuclei with chromosome numbers ranging from 2 to 12 per nucleus and also diploids and polyploids. Because it has been shown that the expression of different genes is proportional to, inversely proportional to, or unaffected by dosage, obviously the existence of heteroploid cells or heteroploid whole individuals of some pathogens increases the degree of variability that can be exhibited by these pathogens. Heterploidy has been observed repeatedly in fungi and has been shown to affect the growth rate, spore size and rate of spore production, hyphal color, enzyme activities, and pathogenicity. It has been shown, for example, that some heteroploids, such as diploids of the normally haploid fungus *Verticillium alboatrum*, the cause of wilt in cotton, lose the ability to infect cotton plants even when derived from highly virulent haploids. How much of the variability in pathogenicity in nature is due to heteroploidy is still unknown.

Sexual-like Processes in Bacteria and Horizontal Gene Transfer

New biotypes of bacteria seem to arise with varying frequency by means of at least three sexual-like processes (Fig. 4-6). It is probable that similar processes occur in mollicutes. (1) **Conjugation** occurs when two compatible bacteria come in contact with one another and a small portion of the chromosome or plasmid from one bacterium is transferred to the other through a conjugation bridge or pilus. (2) In **transformation**, bacterial cells are transformed genetically by absorbing and incorporating in their own cells genetic material secreted by, or released during rupture of, other bacteria. (3) In **transduction**, a bacterial virus (phage) transfers genetic material from the bacterium in which the phage was produced to the bacterium it infects next. The transfer of genetic information in this manner is not always limited to members of the same species or even genus (**vertical inheritance**). For example, gram-negative bacteria can transmit genetic material readily across species; *Agrobacterium* transmits genes across kingdom barriers to plants. Such events are called **horizontal gene transfers.**

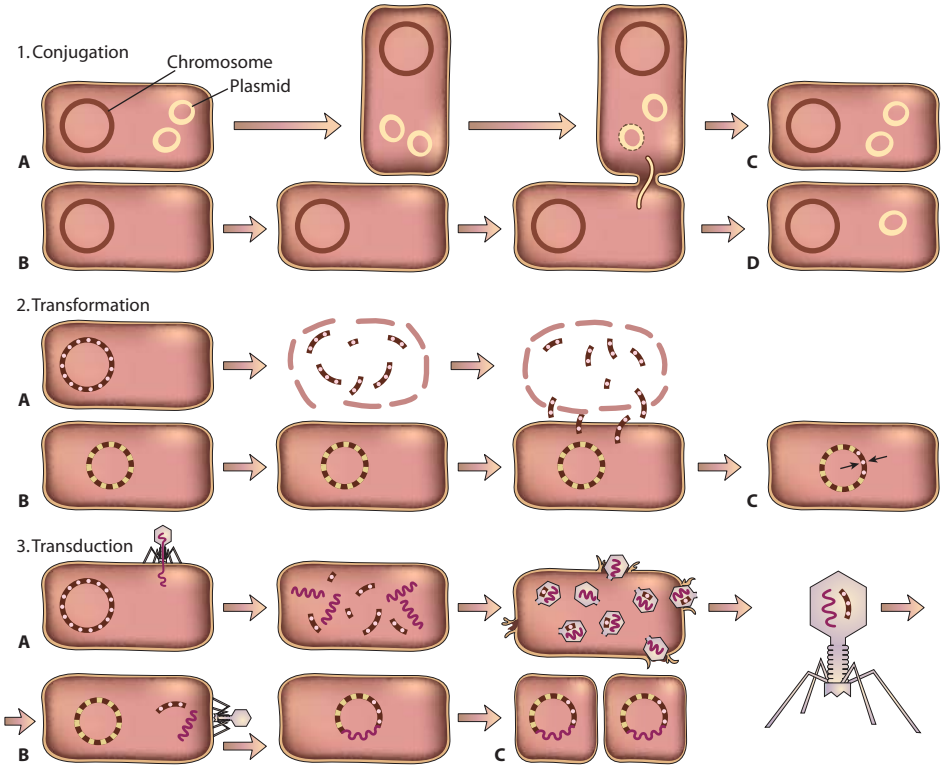

FIGURE 4-6 Mechanisms of variability in bacteria through sexual-like processes.

Genetic Recombination in Viruses

When two strains of the same virus are inoculated into the same host plant, one or more new virus strains are recovered with properties (virulence, symptomatology, and so on) different from those of either of the original strains introduced into the host. The new strains probably are recombinants, although their appearance through mutation, not hybridization, cannot always be ruled out. In multipartite viruses consisting of two, three, or more nucleic acid components, new virus strains may also arise in host plants or vectors from recombination of the appropriate components of two or more strains of such viruses.

Loss of Pathogen Virulence in Culture

The virulence of pathogenic microorganisms toward one or all of their hosts often decreases when the pathogens are kept in culture for relatively long periods of time or when they are passed one or more times through different hosts. If the culturing of the pathogen is prolonged sufficiently, the pathogen may lose virulence completely. Such partial or complete loss of virulence in pathogens is sometimes called **attenuation**, and it has been shown to occur in bacteria, fungi, and viruses. Pathogens that have experienced partial or complete loss of virulence in culture or in other hosts are often capable of regaining part or all of their virulence if they are returned to their hosts under proper conditions. Sometimes, however, the loss of virulence may be irreversible. "Loss" of virulence in culture, or in other hosts, seems to be the result of selection of individuals of less virulent or avirulent pathogen strains that happen to be capable of growing and multiplying in culture, or in the other host, much more rapidly than virulent ones. After several transfers in culture or the other hosts, such attenuated individuals largely, or totally, overtake and replace the virulent ones in the total population so that the pathogen is less virulent or totally avirulent. On reinoculation of the proper host, isolates in which the virulent individuals have been totally replaced by avirulent ones continue to be avirulent, and therefore loss of pathogenicity is irreversible. However, on reinoculation of the proper host with isolates in which at least some virulent individuals survived through the transfers in culture or the other host, the few surviving virulent individuals infect the host and multiply, often in proportion to their virulence. The virulent individuals increase in number with each subsequent inoculation while at the same time nonvirulent individuals are reduced or eliminated with each reinoculation.

STAGES OF VARIATION IN PATHOGENS

The entire population of a particular organism on the earth, e.g., a fungal pathogen, has certain morphological and other phenotypic characteristics in common and makes up the **species** of pathogen, such as *Puccinia graminis*, the cause of stem rust of cereals. Some individuals of this species, however, attack only wheat, barley, or oats, and these individuals make up groups that are called **varieties** or **special forms** (*formae specialis*) such as *P. graminis* f. sp. *tritici* or *P. graminis tritici*, *P. graminis hordei*, and *P. graminis avenae* (Table 4-1). Even within each special form, however, some individuals attack some of the varieties of the host plant but not others, some attack another set of host plant varieties, and so on, with each group of such individuals making up a race. Thus, there are more than 200 races of *P. graminis tritici* (race 1, race 15, race 59, and so on). Occasionally, one of the offspring of a race can suddenly attack a new variety or can cause severe symptoms on a variety that it could barely infect before. This individual is called a **variant**. The identical individuals produced asexually by the variant make up a **biotype**. Each race consists of one or several biotypes (race 15A, 15B, and so on).

The appearance of new pathogen biotypes may be very dramatic when the change involves the host range of the pathogen. If the variant has lost the ability to infect a plant variety that is widely cultivated, this pathogen simply loses its ability to procure a livelihood for itself and will die without even making its existence known to us. If, however, the change in the variant pathogen enables it to infect a plant variety cultivated because of its resistance to the parental race or strain, the variant individual, being the only one that can survive on this plant variety, grows and multiplies on the new variety without any competition and soon produces large populations that spread and destroy the heretofore resistant variety. This is the way the resistance of a plant variety is said to be "broken down," although it was the change in the pathogen, not the host plant, that brought it about.

TYPES OF PLANT RESISTANCE TO PATHOGENS

Plants are resistant to certain pathogens because they belong to taxonomic groups that are outside the host range of these pathogens (nonhost resistance), because they possess genes for resistance (R genes) directed against the avirulence genes of the pathogen (true, race-specific, cultivar-specific, or gene-for-gene resistance), or because, for various reasons, the plants escape or tolerate infection by these pathogens (apparent resistance).

Each kind of plant, e.g., potato, corn, or orange, is a host to a small and different set of pathogens that make up a small proportion of the total number of known plant pathogens. In other words, each kind of plant is a nonhost to the vast majority of known plant pathogens. Nonhosts are completely resistant to pathogens of other plants, usually even under the most favorable conditions for disease development (nonhost resistance). The same species of plants, however, that are nonhosts to most

TABLE 4-1
Stages of Variation in Plants and Pathogens and Characteristics by Which They Are Distinguished

Distinguishing characteristics	Fungi	Bacteria	Viruses	Nematodes	Plants
Morphology and biochemistry	Genus	Genus	Genus (formerly group)	Genus	Genus
	↓	↓	↓	↓	↓
Morphology and biochemistry	Species	Species	Virus name (species)	Species	Species
	↓	↓	↓	↓	↓
Host	Variety or special form	Variety or pathovar	Type[a]	Race or	Variety or cultivar
	↓	↓	↓		
Differential varieties or symptoms	Race	Race	Strain	biotype or pathotype	↓
	↓	↓	↓		
Localized field population	Isolate	Isolate	Isolate	or strain	
	↓	↓	↓	↓	↓
Clonal population	Single spore-derived biotype	Single colony-derived strain	Single local lesion isolate	Individual nematode	Clone

[a]Sometimes strain is used instead of type.

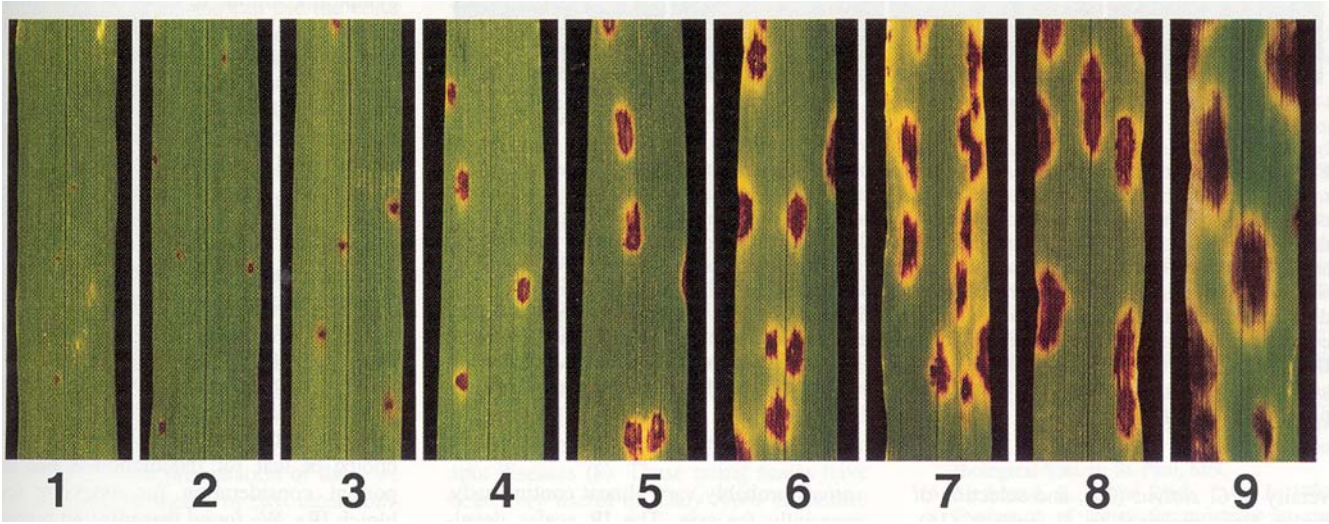

FIGURE 4-7 An infection rating scale of barley seedling leaves inoculated with the same isolate of the spot blotch fungus *Cochliobolus sativus*. Seedlings 1, 2, and 3 indicate low compatibility between hosts and the pathogen, whereas seedlings 4 and 5 show intermediate compatibility and seedlings 6, 7, 8, and 9 show high compatibility (susceptibility). [Photograph courtesy of Fetch and Steffenson (1999). *Plant Dis.* **83**, 213–217.]

pathogens are susceptible, to a lesser or greater extent, to their own pathogens. Moreover, each plant species exhibits specific susceptibility toward each of its own pathogens while it exhibits complete or nonhost resistance to all other pathogens (Figs. 4-4 and 4-5).

Even within a species of plant that is susceptible to a particular species of pathogen, however, there is considerable variation in both the susceptibility of the various plant cultivars (varieties) toward the pathogen (Figs. 4-7 and 4-8) and the virulence of the various pathogen races toward the plant variety. The genetics of such host–pathogen interactions are of considerable biological interest and of the utmost importance in developing disease control strategies through breeding for resistance.

The variation in susceptibility to a pathogen among plant varieties is due to different kinds and, perhaps, different numbers of genes for resistance that may be present in each variety. The effects of individual resistance genes vary from large to minute, depending on the importance of the functions they control. A variety that is very susceptible to a pathogen isolate obviously has no effective genes for resistance against that isolate. The same variety, however, may be resistant to another pathogen isolate obtained from infected plants of another variety.

	Pathogen isolate	
	1	2
Plant variety	Susceptible	Resistant

Lack of susceptibility to the second isolate would indicate that the plant variety, which had no genes for resistance against the first pathogen isolate, has one or more genes for resistance against the second isolate. If the same plant variety is inoculated with more pathogen isolates, obtained from still different plant varieties, it is possible that the variety would be susceptible to some of them but not susceptible (and thus would be resistant) to the other isolates. The latter case would again show that the variety has one or more genes for resistance against each of the isolates to which it is resistant. Although the resistance against some of the isolates might be the result of the same genes for resistance in the variety, it is likely that the variety also contains several genes for resistance, each specific against a particular pathogen isolate.

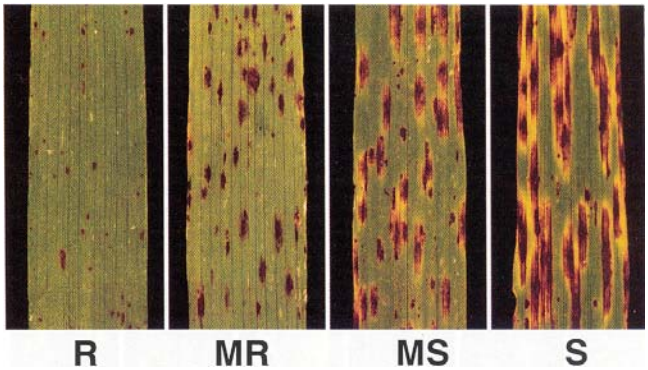

FIGURE 4-8 An infection response rating scale for leaves of adult barley plants inoculated with the same isolate of the spot blotch fungus *Cochliobolus sativus*. Rankings are R, resistant; MR, moderately resistant; MS, moderately susceptible; and S, susceptible. [Photograph courtesy of Fetch and Steffenson (1999). *Plant Dis.* **83**, 213–217.]

True Resistance

Disease resistance that is controlled genetically by the presence of one, a few, or many genes for resistance in the plant is known as true resistance. In true resistance, the host and the pathogen are more or less incompatible with one another, either because of lack of chemical recognition between the host and the pathogen or because the host plant can defend itself against the pathogen. The various defense mechanisms are either already present or are activated in response to infection by the pathogen. There are two kinds of true resistance: partial, also called quantitative, polygenic, or horizontal resistance and R gene resistance, also called race specific, monogenic, or vertical resistance.

Partial, Quantitative, Polygenic, or Horizontal Resistance

All plants have a certain, but not always the same, level of possibly unspecific resistance that is effective against each of their pathogens. Such resistance is sometimes called partial, race nonspecific, general, quantitative, polygenic, adult-plant, field, or durable resistance, but in the past it was referred to most commonly as horizontal resistance.

Partial resistance is probably controlled by several genes, thereby the name **polygenic** or **multigene resistance**. There are, however, several examples of quantitative and nonrace-specific resistance that are determined by single genes, often R gene homologs. Also, in many cases where genetic analyses were performed, a limited number of genes, usually fewer than four to five, often one or two, are sufficient to explain most of the resistance. In many cases, one of these genes alone may be rather ineffective against the pathogen and may play a minor role in the total horizontal resistance (**minor gene resistance**). The several genes involved in partial resistance seem to exert their influence by controlling the numerous steps of the physiological processes in the plant that provide the materials and structures that make up the defense mechanisms of the plant. The partial resistance of a plant variety toward all races of a pathogen may be somewhat greater, or smaller, than those of other varieties toward the same pathogen (Figs. 4-7 and 4-8), but the differences are usually small and insufficient to routinely distinguish varieties (**nondifferential resistance**). In addition, partial resistance is affected by and may vary considerably more than R gene resistance under different environmental conditions. Generally, partial resistance does not protect plants from becoming infected. Instead it slows down the development of individual infection loci on a plant, thereby slowing down the spread of the disease and the development of epidemics in the field (Figs. 4-7–4-9). Some degree of partial resistance is present in plants regardless of whether monogenic resistance is present. However, although it is clear that partial resistance is inherited quantitatively, it is believed that the individual genes contributing to "partial" resistance may, in fact, be qualitatively identical to the genes of monogenic resistance.

R Gene Resistance, Race-Specific, Monogenic, or Vertical Resistance

Many plant varieties are quite resistant to some races of a pathogen while they are susceptible to other races of the same pathogen. In other words, depending on the race of the pathogen used to infect a variety, the variety may appear strongly resistant to one pathogen race and susceptible to another race (**race specific**) under a variety of environmental conditions. Such resistance differentiates clearly between races of a pathogen, as it is effective against specific races of the pathogen and ineffective against others (Figs. 4-9 and 4-10). Such resistance is

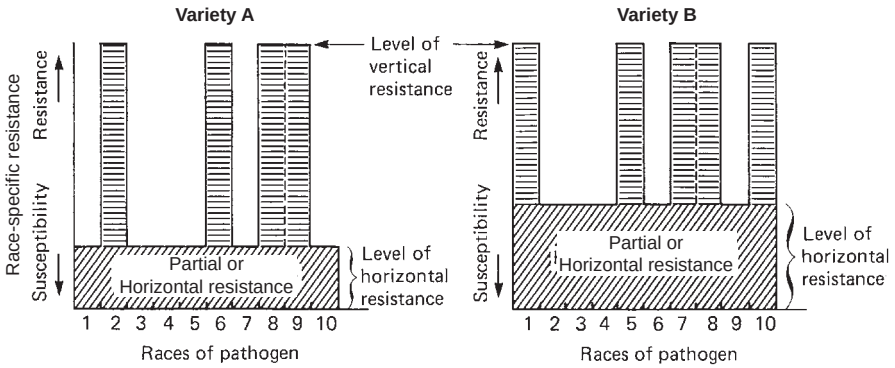

FIGURE 4-9 Levels of horizontal and vertical resistance of two plant varieties toward 10 races of a pathogen. [After Vanderplank (1984).]

FIGURE 4-10 *Brassica napus* plants following inoculation with an isolate of *turnip mosaic virus*. (Left) Susceptible. (Right) Resistant by a single dominant (R) gene. [Photograph courtesy of Walsh and Jenner (2002).]

sometimes called strong, major, race-specific, qualitative, or differential resistance, but it was more commonly referred to in the past as **vertical resistance**.

Race-specific resistance is always controlled by one or a few genes (thereby the names **monogenic** or **oligogenic resistance**). These genes, referred to as R genes, control a major step in the recognition of the pathogen by the host plant and therefore play a major role in the expression of resistance. In the presence of race-specific resistance, the host and pathogen appear incompatible (Fig. 4-9). The host may respond with a hypersensitive reaction, may appear immune, or may inhibit pathogen reproduction. Often, race-specific resistance inhibits the initial establishment of pathogens that arrive at a field from host plants that lack, or have different, major genes for resistance. Race-specific resistance inhibits the development of epidemics by limiting the initial inoculum or by limiting reproduction after infection.

Complete resistance may be provided by a single resistance gene. Often, it is desirable to combine, or **pyramid**, more than one resistance gene (R1R2, R1R3, R1R2R3) in the same plant, which then is resistant to all the pathogen races to which each of the genes provides resistance. A plant species may have as many as 20 to 40 resistance genes against a particular pathogen, although each variety may have only one or a few of these genes. For example, wheat has 20 to 40 genes for resistance against the leaf rust fungus *Puccinia recondita*, barley has a similar number of genes against the powdery mildew fungus *Erysiphe graminis hordei*, and cotton has almost as many against the bacterium *Xanthomonas campestris* pv. *malvacearum*. Each gene for resistance, such as R2, makes the plant resistant to all the races of the pathogen that contain the corresponding gene for avirulence. This pathogen race and its avirulence gene (A2), however, are detected because the pathogen attacks plants that lack the particular gene for resistance (R2).

Whether partial or race specific, true resistance is generally controlled by genes located in the plant chromosomes in the cell nucleus. There are, however, several plant diseases in which resistance is controlled by genetic material contained in the cytoplasm of the cell. Such resistance is sometimes referred to as **cytoplasmic resistance**. The two best-known cases of cytoplasmic resistance occur in the southern corn leaf blight caused by *Bipolaris (Helminthosporium) maydis* and the yellow leaf blight caused by *Phyllosticta maydis*. Resistance in these is conferred by the lack of a gene in mitochondria of normal cytoplasm of various types of corn that encodes a receptor for the host-specific toxin produced by each pathogen. The presence of such a gene in mitochondria of Texas male-sterile cytoplasm makes all corn lines with Texas male-sterile cytoplasm susceptible to these pathogens.

Varieties with race-specific (monogenic or oligogenic) resistance generally show complete resistance to a specific pathogen under most environmental conditions, but a single or a few mutations in the pathogen may produce a new race that may infect the previously resistant variety. On the contrary varieties with partial (polygenic) resistance are less stable and may vary in their reaction to the pathogen under different environmental conditions, but a pathogen will have to undergo many more mutations to completely break down the resistance of the host. As a rule, a combination of major and minor genes for resistance against a pathogen is the most desirable makeup for any plant variety.

Apparent Resistance

In any area and almost every year, limited or widespread plant disease epidemics occur on various crop plants. Under certain conditions or circumstances, however, some very susceptible plants or varieties of these crops may remain free from infection or symptoms and thus appear resistant. The apparent resistance to disease of plants known to be susceptible is generally a result of disease escape or tolerance to disease.

Disease Escape

Disease escape occurs whenever genetically susceptible plants do not become infected because the three factors necessary for disease (susceptible host, virulent pathogen, and favorable environment) do not coincide and interact at the proper time or for sufficient duration. Plants may, for example, escape disease from soil-

borne pathogens because their seeds germinate faster or their seedlings harden earlier than others and before the temperature becomes favorable for the pathogen to attack them. Some plants escape disease because they are susceptible to a pathogen only at a particular growth stage (young leaves, stems, or fruits; at blossoming or fruiting; at maturity and early senescence); therefore, if the pathogen is absent or inactive at that particular time, such plants avoid becoming infected. For example, young tissues and plants are infected and affected much more severely by *Pythium*, powdery mildews, and most bacteria and viruses than older ones. However, fully grown, mature, and senescent plant parts are much more susceptible to certain other pathogens, such as *Alternaria* and *Botrytis*, than their younger counterparts. Plants may also escape disease because of the distance between fields, the number and position of plants in the field, the spacing of plants in a field, and so on.

In many cases, plants escape disease because they are interspersed with other types of plants that are insusceptible to the pathogen and because the amount of inoculum that reaches them is much less than if they were in monocultural plantations; because their surface hairs and wax repel water and pathogens suspended in it; because their growth habit is too erect or otherwise unfavorable for pathogen attachment and germination; or because their natural openings, such as stomata, are at a higher level than the rest of the leaf surface or open too late in the day, by which time the leaves are dry and the germ tubes of spores, such as of *Puccinia graminis*, have desiccated. In plant diseases in which pathogens penetrate primarily through wounds caused by heavy winds and rain, dust storms, and insects, lack of such wounds allows disease escape. Also, plants that are unattractive or resistant to the vector of a pathogen escape infection by that pathogen.

Factors that affect the survival, infectivity, multiplication, and dissemination of the pathogen are also likely to allow some plants to escape disease. Such factors include the following: absence or poor growth of the pathogen at the time the susceptible plant stage is available; destruction or weakening of the pathogen by hyperparasites or by antagonistic microflora at the place of production or at the infection court; misdirection to or trapping of the pathogen by other plants; and lack of pathogen dissemination because winds, rain, or vectors are absent.

Several environmental factors play crucial roles in plant disease escape in almost every location. Temperature, for example, determines the geographical distribution of most pathogens, and plants growing outside the range of that temperature escape disease from such pathogens. Most commonly, however, plant disease escape increases in temperature ranges that favor plant growth much more than they do the growth of the pathogen. For example, many plants escape disease from *Pythium* and *Phytophthora* if the temperature is high and the soil moisture low, whereas some low temperature crops, such as wheat, escape similar diseases from *Fusarium* and *Rhizoctonia* if the temperature is low. Temperatures outside certain ranges inhibit the sporulation of fungi as well as spore germination and infection, thereby increasing the chances for disease escape. Low temperatures also reduce the mobility of many insect vectors or pathogens, allowing more plants to escape disease.

Lack of moisture caused by low rainfall or dew or low relative humidity is probably the most common cause of disease escape in plants. Plants in most dry areas or during dry years remain generally free of apple scab, late blight, most downy mildews, and anthracnoses because these diseases require a film of water on the plant or high relative humidity in almost every stage of their life cycle. Similarly, in dry soils such diseases as clubroot of crucifers and damping off induced by *Pythium* and *Phytophthora* are quite rare because such soils inhibit the production and activity of the motile spores of these pathogens. However, with some diseases, such as common scab of potato caused by *Streptomyces scabies*, plants escape disease in irrigated or wet soils because the plants can defend themselves better in the absence of water stress and because these pathogens are lysed or otherwise inhibited by microorganisms favored by high moisture. Many trees are also in a better position to defend themselves and to escape damage by the canker-causing fungus *Leucostoma* sp. (*Cytospora* sp.) in years in which sufficient rainfall or irrigation provides adequate soil moisture in late summer and early fall.

Some other environmental factors also allow plants to escape disease. For example, wind may increase disease escape by blowing from the wrong direction at the right time, thus carrying spores and vectors away from the crop plants, and by drying up plant surfaces quickly before the pathogen has time to germinate and infect. Also, soil pH increases disease escape in a few diseases, e.g., in crucifers from *Plasmodiophora brassicae* at high pH and in potatoes from *Streptomyces scabies* at low pH, in both cases because the particular pH inhibits survival and growth of the pathogen.

In general, many plant diseases are present some years in some areas and absent on the same kinds of plants in other years or in nearby areas. This suggests that in these areas or years the plants remain free of disease not because they are resistant, but because they escape disease. Earliness is often bred into many wheat and potato varieties to help them escape disease from the rusts and the late blight, respectively. Lateness, rapid

growth, resistance to bruising, unattractiveness to vectors, and tolerance to low temperatures are also often bred into crop varieties to help them escape specific diseases. These and many other characteristics, of course, are those that make up horizontal resistance. It is true that there is a wide common area between horizontal resistance and disease escape in which the one leads to the other or the two appear identical. Escape from disease depends on environmental conditions, as well as on heritable characteristics in the host and the pathogen, and is often entirely controlled by the environment. Escape from disease, moreover, is a manageable quality, and farmers, through many of the most common cultural practices, actually aim at helping plants escape disease. Such practices include using disease-free, vigorous seed, choosing the proper soil, planting date, depth of sowing, and distance between plants and between fields, utilizing proper crop rotation, sanitation (rouging, pruning, and so on), interplantings, and multilines, attending to insect and vector control, and several others.

Tolerance to Disease

Tolerance to disease is the ability of plants to produce a good crop even when they are infected with a pathogen. Tolerance results from specific, heritable characteristics of the host plant that allow the pathogen to develop and multiply in the host while the host, either by lacking receptor sites for or by inactivating or compensating for the irritant excretions of the pathogen, still manages to produce a good crop. Tolerant plants are, obviously, susceptible to the pathogen, but they are not killed by it and generally show little damage. The genetics of tolerance to disease are not understood; neither is its relationship, if any, to horizontal resistance. Tolerant plants, whether because of exceptional vigor or a hardy structure, probably exist in most host–parasite combinations. Tolerance to disease is observed most commonly in many plant–virus infections in which mild viruses, or mild strains of virulent viruses, infect plants such as potato and apple systemically and yet cause few or no symptoms and have little discernible effect on yield. Generally, however, although tolerant plants produce a good crop even when they are infected, they produce an even better crop when they are not infected.

GENETICS OF VIRULENCE IN PATHOGENS AND OF RESISTANCE IN HOST PLANTS

Infectious plant diseases are the result of the interaction of at least two organisms, the host plant and the pathogen. The properties of each of these two organisms are governed by their genetic material, the DNA, which is organized in numerous segments making up the genes.

It has been known for more than a century that the host reaction, i.e., the degree of susceptibility or resistance to various pathogens, is an inherited characteristic. This knowledge has been used quite effectively in breeding and distributing varieties resistant to pathogens causing particular diseases. The ability of pathogens to inherit their infection type, however, i.e., the degree of pathogen virulence or avirulence, has been studied intensively only since the 1940s. It is now clear that pathogens consist of a multitude of races, each different from others in its ability to attack certain varieties of a plant species but not other varieties. Thus, when a variety is inoculated with two appropriately chosen races of a pathogen, the variety is susceptible to one race but resistant to the other. Conversely, when the same race of a pathogen is inoculated on two appropriately chosen varieties of a host plant, one variety is susceptible while the other is resistant to the same pathogen (Table 4-2). This clearly indicates that, in the first case, one race possesses a genetic characteristic that enables it to attack the plant, while the other race does not, and, in the second case, that the one variety possesses a genetic characteristic that enables it to defend itself against the pathogen so that it remains resistant, while the other variety does not. When several varieties are inoculated separately with one of several races of the pathogen, it is again noted that one pathogen race can infect a certain group of varieties, another race can infect another group of varieties, including some that can and some that cannot be infected by the previous race, and so on (Table 4-2).

Studies of the inheritance of resistance versus susceptibility in plants prove that single genes control resistance and their absence allows susceptibility. Studies of the inheritance of avirulence versus virulence in pathogens prove that single genes control avirulence and their absence allows virulence. Studies of their interac-

TABLE 4-2

Possible Reactions of Two (Left) and Four (Right) Varieties of a Plant to Two (Left) and Four (Right) Races of a Pathogen[a]

Plant variety	Pathogen race 1	Pathogen race 2	Plant variety	Pathogen race 1	Pathogen race 2	Pathogen race 3	Pathogen race 4
A	–	+	A	–	+	+	+
B	+	–	B	+	–	–	+
			C	–	+	–	+
			D	+	–	+	–

[a]Plus signs indicate susceptible (compatible reaction, infection); minus signs indicate resistant (incompatible reaction, noninfection).

tions prove that R genes in the plant are specific for avr genes in the pathogen. Thus, varieties possessing certain genes for resistance react differently against the various pathogen races and their genes for avirulence. The progeny of these varieties react to the same pathogens in exactly the same manner as the parent plants, indicating that the property of resistance or susceptibility against a pathogen is genetically controlled (inherited). Similarly, the progeny of each pathogen causes on each variety the same effect that was caused by the parent pathogens, indicating that the property of virulence or avirulence of the pathogen on a particular variety is also genetically controlled (inherited).

It thus appears that, under favorable environmental conditions, the outcome — infection (susceptibility) or noninfection (resistance) — in each host–pathogen combination is predetermined by the genetic material of the host and of the pathogen. The number of genes determining resistance or susceptibility varies from plant to plant, as the number of genes determining virulence or avirulence varies from pathogen to pathogen. In most host–pathogen combinations the number of genes involved and what they control are not yet known. In some diseases, however, particularly those caused by oomycetes, such as potato late blight, fungi, such as apple scab, powdery mildews, tomato leaf mold, and cereal smuts and rusts, and also in several viral and bacterial diseases of plants, considerable information regarding the genetics of host–pathogen interactions is available.

The Gene-for-Gene Concept

The coexistence of host plants and their pathogens side by side in nature indicates that the two have been evolving together. Changes in the virulence of the pathogens appear to be continually balanced by changes in the resistance of the host, and vice versa. In that way, a dynamic equilibrium of resistance and virulence is maintained, and both host and pathogen survive over considerable periods of time. The stepwise evolution of virulence and resistance can be explained by the **gene-for-gene concept**, according to which for each gene that confers virulence to the pathogen there is a corresponding gene in the host that confers resistance to the host, and vice versa.

The gene-for-gene concept was first proved in the case of flax and flax rust, but it has since been shown to operate in many other rusts, in the smuts, powdery mildews, apple scab, late blight of potato, and other diseases caused by fungi, as well as in several diseases caused by bacteria, viruses, parasitic higher plants, nematodes, and even insects. Generally, but not always, in the host the genes for resistance are dominant (R), whereas genes for susceptibility, i.e., lack of resistance, are recessive (r). In the pathogen, however, genes for avirulence, i.e., inability to infect, are usually dominant (A) whereas genes for virulence are recessive (a). Thus, when two plant varieties, one carrying the gene for resistance R to a certain pathogen and the other lacking the gene R for resistance, i.e., carrying the gene for susceptibility (r) to the same pathogen, are inoculated with two races of the pathogen, one of which carries the gene for avirulence A against the resistance gene R and the other of which carries the gene for virulence (a) against the resistance gene R, the gene combinations and reaction types shown in Table 4-3 and Fig. 4-11 are possible.

Each gene in the host can be identified only by its counterpart gene in the pathogen, and vice versa. Of the four possible gene combinations, only the AR interaction is incompatible (resistant), i.e., the host has a certain gene for resistance (R) that recognizes the corresponding specific gene for avirulence (A) of the pathogen. In the Ar combination, infection results because the host lacks genes for resistance (r) and so the pathogen can attack it with its other genes for virulence (after all, it is a pathogen on this host). In aR, infection results because although the host has a gene for resistance, the pathogen lacks the gene for avirulence that is recognized specifically by this particular gene for resistance and therefore no defense mechanisms (resistance) are activated. Finally, in the ar interaction, infection results because the plant has no resistance (r) and the pathogen, being a pathogen and therefore virulent (a), attacks it.

It is thought that genes for resistance appear and accumulate first in hosts through evolution and that they coexist with nonspecific genes for pathogenicity which evolve in pathogens. Genes for pathogenecity exist in pathogens against all host plants that lack

TABLE 4-3
Quadratic Check of Gene Combinations and Disease Reaction Types in a Host–Pathogen System in Which the Gene-for-Gene Concept for One Gene Operates[a]

	Resistance or susceptibility genes in the plant	
Virulence or avirulence genes in the pathogen	R (resistant) dominant	r (susceptible) recessive
A (avirulent) dominant	AR (–)	Ar (+)
a (virulent) recessive	aR (+)	ar (+)

[a]Minus signs indicate incompatible (resistant) reactions and therefore no infection. Plus signs indicate compatible (susceptible) reactions and therefore infection develops.

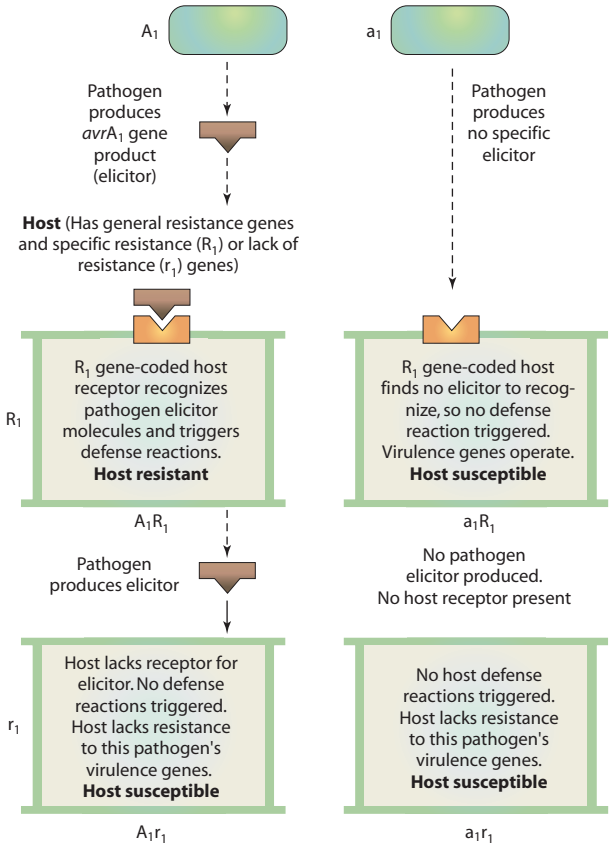

FIGURE 4-11 Basic interactions of pathogen avirulence (A)/virulence (a) genes with host resistance (R)/susceptibility (r) genes in a gene-for-gene relationship and final outcomes of the interactions.

specific resistance. When a specific gene for resistance appears in or is bred into the host, the gene enables the host to recognize the product of a particular gene for virulence in the pathogen. That pathogen gene is then thought of as the "avirulence" gene (*avr*A) of the pathogen that corresponds to the plant resistance gene R. The change in the function of the pathogen gene is because subsequent recognition of the *avr*A gene product (the elicitor molecule) by the receptor coded by the R gene triggers the hypersensitive response reaction in the plant that keeps the plant resistant. A new gene for virulence that attacks the existing gene for resistance appears by mutation of an existing avirulence gene, which then avoids gene-for-gene recognition, and the resistance of the host breaks down. Plant breeders then introduce another gene for resistance (R) in the plant, which recognizes the protein of the new gene for virulence of the pathogen and extends the resistance of the host beyond the range of the new gene for virulence in the pathogen. This produces a variety that is resistant to all races that have an avirulence gene corresponding to the specific gene for resistance until another gene for virulence appears in the pathogen. When a variety has two or more genes for resistance (R_1, R_2, . . .) against a particular pathogen, it means that each corresponds to one, two, or more genes of former virulence (and now avirulence) in the pathogen (a_1, a_2, . . .), each of which, once recognized by one of the genes for resistance in the host, subsequently functions as an avirulence gene. The gene combinations, and disease reaction types, of hosts and pathogens with two genes for resistance or virulence in corresponding loci, respectively, are shown in Table 4-4.

Table 4-4 makes clear several points. First, susceptible (r_1r_2) plants lacking genes for resistance are attacked by all races of the pathogen, regardless of the virulence (aa) or avirulence genes (A_1A_2) carried by the pathogen. Second, pathogen races or individuals designated a_1a_2, i.e., which lack genes for avirulence (A_1A_2) for each gene for resistance of the host (R_1R_2), can infect all plants that have any combination of these genes (R_1R_2, R_1r_2, r_1R_2), as the a_1a_2 pathogen produces no elicitor molecules capable of triggering the host defense response. When a pathogen has one of the two genes for virulence (a_1 or a_2), i.e., it lacks one of the two genes for avirulence (A_1 or A_2), then it can infect plants that have the corresponding gene for resistance (R_1 or R_2, respectively) but not plants that have a gene for resistance corresponding to a gene for avirulence in the pathogen (e.g., pathogen with genes A_1a_2 infects plant with r_1R_2 but not those with R_1r_2 because R_1 can recognize the avr gene A_1 and triggers defenses against it).

The gene-for-gene concept has been demonstrated repeatedly, and both pathogen avirulence genes and plant resistance genes have been isolated. Plant breeders apply the gene-for-gene concept every time they incorporate a new resistance gene into a desirable variety that becomes susceptible to a new strain of the pathogen. With the diseases of some crops, new resistance genes must be found and introduced into old varieties at relatively frequent intervals, whereas in others a single gene confers resistance to the varieties for many years.

TABLE 4-4
Complementary Interaction of Two Host Genes for Resistance and the Corresponding Two Pathogen Genes for Virulence and Their Disease Reaction Types

		Resistance (R) or susceptibility (r) genes in the plant			
		R_1R_2	R_1r_2	r_1R_2	r_1r_2
Virulence (a) or avirulence (A) genes in the pathogen	A_1A_2	–	–	–	+
	A_1a_2	–	–	+	+
	a_1A_2	–	+	–	+
	a_1a_2	+	+	+	+

The Nature of Resistance to Disease

A microorganism is pathogenic, i.e., it is a pathogen, because it has the genetic ability to infect another organism and to cause disease. Either a plant is immune to a pathogen, i.e., it is not attacked by the pathogen even under the most favorable conditions, or it may show various degrees of resistance ranging from near immunity to complete susceptibility. Resistance may be conditioned by a number of internal and external factors that operate to reduce the chance and degree of infection. The first step in any infection is recognition of the host by the pathogen and perhaps the opposite, some type of recognition of the pathogen by the host. Therefore, absence of a recognition factor(s) in the host could help it avoid infection by a particular pathogen. Generally, any heritable characteristic of the plant that contributes to localization and isolation of the pathogen at the points of entry, to reduction of the harmful effects of toxic substances produced by the pathogen, or to inhibition of the reproduction and, thereby, further spread of the pathogen, contributes to the resistance of the plant to disease. As a result, in most plant diseases, the pathogen is usually localized after varying degrees of invasion and colonization of host tissues. Indeed, there are only a few diseases in which the pathogen is allowed to advance unchecked throughout the plant and to kill the entire plant. Furthermore, any heritable characteristic that enables a particular variety to complete its development and maturation under conditions that do not favor the development of the pathogen also contributes to resistance (disease escape).

The contribution of genes conditioning resistance in the host seems to consist, primarily, of providing the genetic potential in the plant for the development of one or more of the morphological or physiological characters that contribute to disease resistance (including those described in Chapter 6 in the sections on structural and biochemical defense). The mechanisms by which genes control the physiological processes that lead to disease resistance or susceptibility are not yet clear, but they are, presumably, no different from the mechanisms controlling any other physiological process in living organisms.

It is thought that for the production of an inducible enzyme or of a fungitoxic substance needed for defense, a stimulant (elicitor), either secreted by the pathogen or caused by the activities of a pathogen, reacts with a receptor molecule of a host cell. This then transmits signals to other host cell molecules, activating plant defenses. However, if a pathogen mutant appears that does not secrete the particular elicitor that activates the defense reaction, the pathogen infects the host without opposition and so causes disease. In the latter case, the resistance of the host is said to have broken down, but it is actually bypassed by the pathogen rather than broken down. Other possible, although unproved, ways by which a pathogen could "break down" the resistance of a host are through a mutation in the pathogen that enables it to produce a substance that can react with and neutralize the defensive toxic substance of the host that is directed against the pathogen and through a mutation in the pathogen that would eliminate or block its own receptor site on which the host defensive substance becomes attached. The pathogen then can operate in the presence of that substance and of the defense mechanism that produces it.

Pathogenicity Genes in Plant Pathogens

Genes Involved in Pathogenesis and Virulence by Pathogens

Plant-infecting pathogens possess several classes of genes that are essential for causing disease (pathogenicity genes) or for increasing virulence on one or a few hosts (virulence genes).

Pathogenicity factors encoded by "pathogenicity genes" (*pat*) and "disease-specific genes" (*dsp*) are those involved in steps crucial for the establishment of disease (Fig. 4-12). Such genes include those essential for recognition of host by pathogen, attachment of the pathogen to the plant surface, germination and formation of infection structures on the plant surface, penetration of the host, and colonization of the host tissue. Genes involved in the synthesis and modification of the lipopolysaccharide cell wall of gram-negative bacteria may help condition the host range of the bacteria.

Some plant cell wall-degrading enzymes (e.g., cutinases), some toxins (e.g., victorin and HC toxin), hormones (e.g., indole acetic acid and cytokinin), polysaccharides, proteinases, siderophores, and melanin are produced by pathogens in pathogen–plant interactions in which they are essential for the pathogen to infect and cause disease on its host. In those cases, therefore, such factors function as pathogenicity factors. In other plant–pathogen systems the same compounds are helpful but not essential for disease induction and development. In these cases, these compounds are considered virulence factors. There is almost an unlimited number of virulence factors produced by pathogens. They include, in addition to many cell wall-degrading enzymes, toxins, hormones, and polysaccharides, almost all molecules or structures, e.g., amylases, lipases, signaling molecules such as homoserine lactone exopolysaccharides, and flagella. These compounds or structures may be present on the pathogen surface or

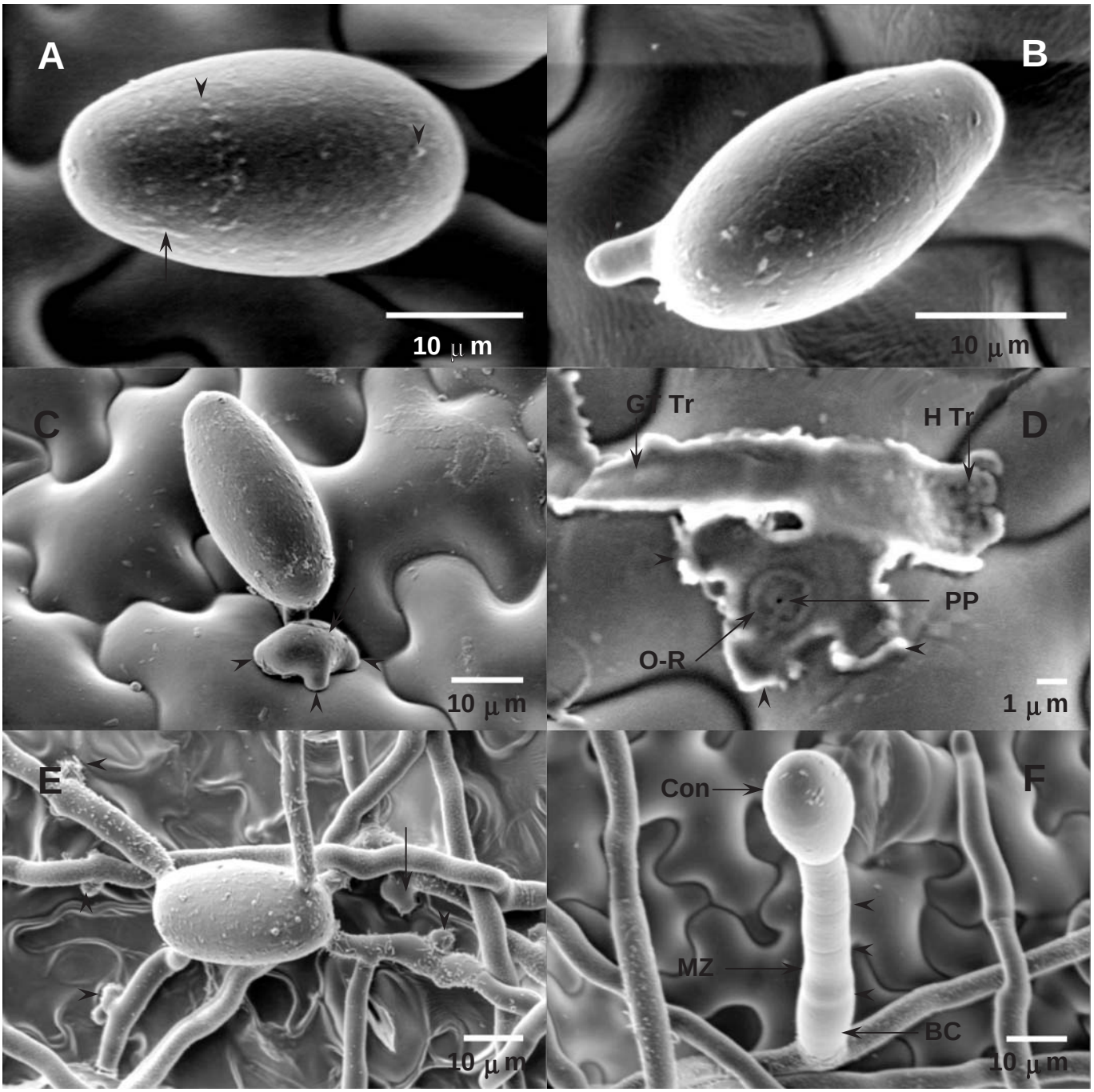

FIGURE 4-12 Electron micrographs of the infection stages of a tomato leaf by a conidium of the powdery mildew fungus *Oidium neolycopersici*. (A) Conidium. (B) Conidium with germ tube. (C) An appressorium forming at the end of the germ tube 10 hours postinoculation. (D) Imprint left on leaf after peeling germ tube and appressorium. A circular hole in the center of the appressorium shows the penetration pore made by the penetration peg. (E) Mycelium and pairs of hyphal appressoria. (F) A conidiophore bearing a conidium. [Photo courtesy S.J. Gurr, from *Can. J. Bot.* 73: (Supp 1), 5632–5639, 1995]

translocated to the extracellular environment of the pathogen and, in a variety of ways, could influence growth of the pathogen in the plant.

Plant pathogens employ diverse strategies to infect their host plants. Depending on the type of pathogen and on the infection process followed by each of them, pathogens utilize various genes that enable them to adhere to their host, form infection structures, penetrate the host, break down host wall macromolecules, produce toxins, neutralize host defenses, obtain

nutrients from the host, move through the host, reproduce in the host, disseminate from host to host, respond to the environment, and so on.

Pathogenicity genes are genes that make a particular (micro)organism a pathogen, i.e., capable of causing disease. Disruption of a pathogenicity gene results in a complete loss or drastic reduction of disease symptoms. It should be noted here that virulence/avirulence genes act on top of the general pathogenicity of the pathogen and, in some cases, may have additional roles in disease. The most important types of pathogenicity genes of the main kinds of plant pathogens are discussed briefly.

Pathogenicity Genes of Fungi

Plant pathogenic fungi utilize a variety of ways and means (chemotropism, thigmotropism) to recognize and adhere to their host plant. Depending on whether the fungi enter the plant through wounds, stomata, or through direct penetration they may need to degrade the cuticle and the cell wall, for which they may need to form specialized structures, such as appressoria. Once inside the plant, the fungus may obtain nutrients without killing cells (biotroph), it may kill cells through its toxins and feed off the contents of dead cells (necrotroph), or it may act as a biotroph in early stages of infection but as a necrotroph later on.

Pathogenicity Genes Controlling Production of Infection Structures

Many fungi produce appressoria that help them penetrate epidermal cells. Appressoria contain glycerol for creating a high turgor pressure that allows the penetration peg to puncture the plant epidermal cells. Appressorial walls of *Magnaporthe grisea* and *Colletotrichum* species contain melanin that prevents glycerol from leaking out. Melanin-deficient mutants are unable to generate turgor pressure and are, therefore, nonpathogenic. Melanin biosynthesis is carried out by at least three structural genes, all of which are essential for pathogenicity of both fungi.

Several genes are involved in appressorial development, which is under both environmental and genetic control. For example, in the rice blast disease, caused by *M. grisea*, several genes have been shown to control appressorial development. One such gene, hydrophobin (mpg1), is essential for appressorial formation and, when disrupted, the fungus not only has reduced pathogenicity, it produces 100 times fewer conidia. Transcription of the mpg1 gene is controlled by three regulatory genes, two of which are also involved in the regulation of nitrogen metabolism. Another gene expressed in spores of *M. grisea* resembles transcription factors, but its disruption leads to the production of defective conidia and impaired appressorium formation, both of which cause loss of pathogenicity. A still different gene, pth11, encodes a protein that is embedded in the cell membrane and apparently enables the fungus to recognize the host surface and to form normal appressoria; disruption of that gene makes the fungus unable to do either.

Pathogenicity Genes Controlling Degradation of Cuticle and of Cell Wall

It is assumed that enzymes that degrade cell walls, cutin, pectin, and other physical structures are essential for pathogenicity. These enzymes, however, are often encoded by multigene families or by more than one gene that are not related, which results in functional redundancy of such enzymes. As a result, the disruption of one such gene does not eliminate pathogenicity of the pathogen because the other genes that encode the same enzyme fill in the need for the enzyme. Functional redundancy among virulence genes appears to be an emerging theme in explaining the degree of severity in many diseases. In addition, cell wall-degrading enzymes through their action often release oligosaccharides and cell wall proteins that can elicit or suppress the defense responses of the host plant. For example, a mutant of the elicitor enzyme xylanase II, the enzymatic activity of which was reduced 1,000-fold, still elicited a defense response in tomato and tobacco.

Cutins. These are hardy polymers that cover most external plant surfaces. They are degraded by cutinases. Cutinases are, most likely, pathogenicity factors for those fungi that need to penetrate the host surface directly. There is a whole family of cutinase multigenes and, therefore, most attempts to prove that they are essential for pathogenicity through gene disruption have been unsuccessful. The cutinase from *Fusarium solani* f. sp. *pisi*, however, when disrupted, led to mutants that had no pathogenicity.

Pectins. These consist of mixtures of primarily polygalacturonic acid with branches of many sugars. They occur in plant cell walls and in middle lamellae. Pectins exist in numerous forms and are degraded by enzymes such as pectin lyase, polygalacturonase, and pectin methylesterase, all of which appear to play a pathogenicity role in some fungi. Pectinases, however, are also encoded by multigene families, and proof of their significance as essential pathogenicity factors is difficult. Nevertheless, disruption of the gene encoding a pectate lyase in *Colletotrichum* sp. produced mutants that had reduced pathogenicity on avocado fruit.

However, disruption of a pectin lyase gene in *Alternaria* sp., *Glomerella* sp., *and Cryphonectria parasitica* had no effect on its pathogenicity, whereas disruption of a pectinase gene in *Botrytis* reduced the pathogenicity of the fungus on tomato and on apple. In a different case, disruption of either the pectin-inducible pectate lyase or the plant-inducible pectate lyase in *F. solani* pv. *pisi* had no effect on the pathogenicity of the fungus. When, however, both pectate lyase genes in *Fusarium* were disrupted at the same time, all mutants showed reduced pathogenicity. In a still different case, insertion and expression of a polygalacturonase gene in a strain of *Aspergillus flavus,* that lacked polygalacturonase, enabled the fungus to produce larger lesions on cotton bolls. Several other types of genes coding for cell wall-degrading enzymes, such as pectinases, glucanases, and xylanases, have been cloned and subsequently disrupted and their effects studied. Most disruptions failed to induce a loss of pathogenicity in the pathogen, but some gave mixed results. Little is known about the role in pathogenesis of cellulases, ligninases, or hemicellulases.

Pathogenicity Genes Controlling Secondary Metabolites

In addition to needing genes for producing infection structures and for degrading structural obstacles, fungal pathogens need genes that will help them overcome the many secondary metabolites plants produce, some of which have antimicrobial properties and help protect the plant against attack. Secondary metabolite compounds produced constitutively are called phytoanticipins, whereas those produced in response to attack by a pathogen are called phytoalexins. Pathogens respond to these chemical defenses of the host plant through genes that help pathogens avoid them, degrade them, alter their physiology, or through other mechanisms.

Phytoanticipins. They include primarily the saponins avenacin and tomatine. Saponins are glycosides with soap-like properties that can disrupt membranes. One saponin, avenacin A-1, is localized in the epidermis of oat roots but not of wheat roots. The fungus *Gaeumannomyces graminis* var. *avena* can infect oats because it has a gene that codes for the enzyme avenacinase, which degrades the saponin. When the avenacinase gene is disrupted, however, the avenacin-less mutants of the fungus fail to infect oats while they can still infect wheat, which does not produce avenacin. Another saponin, α-tomatine, is produced in tomato and has antimicrobial activity against many fungi. The fungus *Septoria lycopersici*, however, carries a gene similar in sequence to the avenacinase gene that encodes the enzyme tomatinase, which degrades the saponin tomatine. Disruption of the tomatinase gene, however, did not reduce the pathogenicity of *Septoria* on tomato, possibly because the fungus has other enzymes that can degrade the saponin. The latter happens in the oat — *Stagonospora avenae* interaction in which the fungus has three genes encoding for enzymes that can degrade the particular saponin.

Cyanogenic Glycosides and Glycosinolates. These compounds are separated in the plant from the enzymes that can degrade them. Upon wounding of a plant, these compounds and their enzymes mingle and interact, producing cyanide, isocyanates, nitriles, and thiocyanates, all toxic against all organisms and also to fungi. Their role, however, in pathogenesis of fungi and how the latter defend themselves, are not known.

Phytoalexins. Phytoalexins have been known for several decades to be produced by plants under attack but few fungal enzymes have been found that degrade them during fungal attack. One such enzyme is pisatin demethylase, which is produced by the fungus *Nectria haematococca* and degrades the pea phytoalexin pisatin. Pisatin demethylase is encoded by one of six such genes of the fungus but disruption of the gene caused only a slight reduction in pathogenicity. However, disruption of one out of four fungal genes that detoxify the phytoalexin maakiain from chickpea resulted in a reduction of pathogenicity, whereas the insertion of additional copies of the same gene in the pathogen isolates resulted in greater disease severity.

Some fungal genes protect the fungus and its pathogenicity even after it is growing inside the plant. Numerous such genes are involved in the efflux and influx of fungal molecules into the plant. Disruption of such a gene in *M. grisea* resulted in loss of pathogenicity. Because the same gene is induced by toxic drugs and by the rice phytoalexin sakuranetin, perhaps it plays a role in the efflux of plant metabolites from the fungus.

Because some fungal pathogenicity genes, when mutated, result in auxotrophic strains, it is apparent that levels of nutrients can affect the ability of fungi to colonize plants. It has been known for many years that auxotrophy is linked to a lack of pathogenicity in the corn smut fungus *Ustilago maydis*, whereas adenine auxotrophs of the apple scab fungus *Venturia inaequalis* are nonpathogenic on apple. Similarly, auxotrophs of *Fusarium sp*. in arginine and of *Stagonospora sp*. in ornithine decarboxylase also lost their ability to cause disease.

Pathogenicity Genes Controlling Fungal Toxins

Some fungi produce toxins that can disrupt host cellular functions or kill cells before or during infection.

Some toxins are nonspecific, i.e., they damage plants not attacked by the pathogen, whereas other toxins are host specific, i.e., they only damage plants that are attacked by the pathogen. The cellular targets of four host-specific fungal toxins, and possible mechanisms of action that lead to programmed cell death of their host plant cells have been studied. The HC toxin acts in the nucleus where it inhibits histone deacetylation, brings about changes in gene expression and prevents synthesis of antifungal compounds by the plant. The *Alternaria alternata* (AAL) toxin inhibits synthesis of the endoplasmic reticulum (ER) enzyme ceramide synthase; it catalyzes the formation of ceramide from phytosphingosine, both of which in animals and probably in plants can alter the signal transduction activity of the protein kinase. The T toxin reacts with the protein Urf13p of the T-cms mitochondria membrane and causes the formation of pores in it, leading to a loss of H^+ and other ions, and to cell death. Finally, victorin inhibits the enzyme glycine decarboxylase of the photorespiratory cycle and leads to the cleavage of RUBISCO, through which products of the oxygenase reaction are exchanged among the chloroplast (Cp), mitochondrion (Mit), and peroxisome (Px), leading to cell death.

Each toxin requires the participation of several genes for its biosynthesis. The genes that control the biosynthesis of toxins are often clustered together. Disruption of toxin genes in *Cochliobolus* shows that a fungus with an altered toxin profile can still be pathogenic. However, disruption of genes involved in the biosynthesis of *A. alternata* host-specific toxins resulted in reduced pathogenicity. The host-specific toxin produced by the wheat tan spot fungus *Pyrenophora tritici-repentis* is essential for pathogenicity of the fungus, as nonpathogenic toxin-minus mutants of the fungus regained their pathogenicity when they were transformed with the gene encoding the toxin.

Trichothecin are toxic metabolites (mycotoxins) produced by several species of the fungus *Gibberella (Fusarium)* and by the fungus *Myrothecium roridum*. Disruption of the gene that controls the first step in trichothecin biosynthesis resulted in reduced pathogenicity on most, but not all, hosts. Up to 11 genes have been found involved in trichothecin biosynthesis and not all the steps have been studied.

Pathogenicity Signaling Genes Used by Plant Pathogenic Fungi

Fungi, like plants and other organisms, use signaling genes that respond to changes in the environment and set off signaling cascades that alter the expression of their genes. Fungal signaling genes include the G-protein-coding genes, mitogen-activated protein (MAP) kinase genes, and cyclic AMP-dependent protein kinase genes. When such signaling genes are disrupted by mutation, the fungus loses all or most pathogenicity and exhibits a loss or reduction in several other processes, such as growth rate, mating, conidia production, and toxin production. Genes that are part of the signal transduction pathways belong to gene families such as the G protein and MAP kinase ones. In the example of *M. grisea*, three G-protein genes and three MAP kinase genes have been cloned and tested through disruption. Several but not all of the resulting mutants lost pathogenicity.

Genes in signaling pathways seem to code for the same amino acid sequences in the various fungi, but the signaling pathways and their interconnections seem to be different in various fungi. As a result, disruption of one of these genes may cause different effects. For example, disruption of the PMK1 gene of *M. grisea* reduced appressoria formation and lost the ability to infect through a wound but had no effect on mycelium and conidia formation. The CMK1 gene from *Colletotrichum lagenarium* could complement a PMK mutant of *M. grisea* and could restore its pathogenicity. Disruption of the CMK1 gene also reduced the appressorial formation and pathogenicity when inoculated through wounds but, in addition, reduced the melanization of appressoria, conidial production, and conidial germination. Disruption of the homologous gene CHK1 of *Cochliobolus heterostrophus* produced mutant strains that had reduced pathogenicity and, in addition, were infertile. Some signaling genes, in addition to controlling pathogenicity, are also involved in the mating processes in fungi. For example, the basidiomycetous fungi *Ustilago maydis* and *U. hordei* are pathogenic on plants only in a dikaryotic state obtained after two complementary strains mate. The gene loci a and b that control recognition and mating also, in an indirect way, control pathogenicity.

Pathogenicity Genes in Plant Pathogenic Bacteria

Plant pathogenic bacteria enter the intercellular spaces of plants through wounds and/or natural openings, such as stomata. Therefore, bacteria do not need to penetrate the plant surface but they must have ways to adhere.

Bacterial Adhesion to Plant Surfaces

Most bacteria do not need adhesion mechanisms except perhaps when they are moving through the xylem and phloem. The crown gall bacterium *Agrobacterium*, however, requires attachment to plant surface receptors as the first step in the transfer of T-DNA and develop-

ment of disease symptoms. The attachment requires three components: a glucan molecule, the synthesis and export of which requires three genes; genes for the synthesis of cellulose; and the *att* region of the bacterial genome that contains several genes for attachment. In addition to these genes, *Agrobacterium* also contains numerous other genes with homology to genes of mammalian pathogens for adhesins and for pilus biosynthesis.

Several other plant pathogenic bacteria also have genes that encode proteins likely to be involved in attachment and aggregation. Thus, *Ralstonia solanacearum, Pseudomonas, Xanthomonas,* and *Xylella* have as many as 35 genes homologous to type IV pili genes, which in *Xanthomonas* and *Pseudomonas* is involved in cell-to-cell aggregation and protection from environmental stress, whereas in *Xylella* type IV pili are necessary for the establishment of an aggregated bacterial population in the turbulent environment of the xylem by adhering to the vessels in conjunction with components such as polysaccharides. *Xylella, Xanthomonas*, and *Ralstonia*, all colonizing plant vessels at some stage of infection, also contain additional adhesin gene homologs and homologs of hemagglutinin-related genes found in many bacteria pathogenic to mammals.

Bacterial Secretion Systems

Secretion systems are essential pathogenicity tools for bacteria because they make possible the translocation of bacterial proteins and other molecules into host plant cells. Five forms of secretion systems are recognized on the basis of the proteins that form them. Type I-SS is present in almost all plant pathogenic bacteria and carries out the secretion of toxins such as hemolysins, cyclolysin, and rhizobiocin. They consist of ATP-binding cassette (ABC) proteins and are involved in the export and import of a variety of compounds through energy provided by the hydrolysis of ATP. Type II-SS is common in gram-negative bacteria and is involved in the export of various proteins, enzymes, toxins, and virulence factors. Proteins are exported in a two-step process: First as unfolded proteins to the periplasm via the Sec pathway across the inner membrane, then as processed and folded proteins through the periplasm and across the outer membrane via an apparatus consisting of 12–14 proteins encoded by a cluster of genes. *Ralstonia* and *Xanthomonas*, which have two type II-SS per cell, use them for secretion outside the bacterium of virulence factors such as pectinolytic and cellulolytic enzymes. *Xylella* and *Agrobacterium* have one type II-SS per cell and, actually, *Agrobacterium* has the genes for only the first step of protein transport across the inner membrane, using type IV-SS for the rest.

Type III-SS is the most important in terms of pathogenicity of the bacteria in the genera *Pseudomonas, Xanthomonas,* and *Ralstonia*. The primary function of type III-SS is the transport of effector proteins across the bacterial membrane and into the plant cell. Genes that encode protein components of the type III-SS apparatus have a two-third similarity at the amino acid level and such genes are called hypersensitive response conserved (Hrc) genes. Genes that encode the transported proteins, especially the surface exposed ones, have only 35% amino acid similarity. Among the effector proteins in *R. solanacearum* are some avr homologs, most of which are similar to *Pseudomonas avr* genes. In addition to *avr* genes, *Pseudomonas, Ralstonia,* and *Xanthomonas* have effector proteins that are similar to ankyrin-related and leucin-rich proteins found in plants, humans, and insects.

Type IV-SS transports macromolecules from the bacterium to the host cell. The proteins transferred are very similar to those responsible for the mobilization of plasmids among bacteria. The *Agrobacterium tumefaciens vir*B operon encodes 11 proteins that form an organized structure and are involved in the transfer of the T-DNA strand from the bacterium to the plant cell cytoplasm. The transporting structure stretches from the bacterial inner membrane through the outer membrane and terminates in a pilus-like structure that protrudes from the bacterial cell. The type V-SS autotransporter is found in *Xylella* and *Xanthomonas* and contains genes that encode surface-associated adhesins. Similar autotransporters exist in mammalian pathogens and are important for adhesion to epithelial cells.

Pathogenicity of Bacterial Enzymes That Degrade Cell Walls

Plant cell walls are composed of three major polysaccharides: cellulose, hemicellulose, and pectins and, in woody and some other plants, lignin. The number of genes encoding cell wall-degrading enzymes varies greatly in the different plant pathogenic bacteria: Soft-rotting erwinias produce a wider range of enzymes able to degrade plant cell wall components than any other plant pathogenic bacteria. The enzymes include pectinases, cellulases, proteases, and xylanases. Pectinases are believed to be the most important in pathogenesis, as they are responsible for tissue maceration by degrading the pectic substances in the middle lamella and, indirectly, for cell death. Four main types of pectin-degrading enzymes are produced, three (pectate lyase (Pel), pectin lyase (Pnl), and pectin methyl esterase (Pme)) with a high (~8.0) pH optimum, and one polygalacturonase, with a pH optimum of ~6. All are present in many forms or isoenzymes, each encoded by

independent genes. For example, *E. chrysanthemi* produces five major Pel groups arranged into two families and at least three minor Pel groups induced preferentially in plant tissue and arranged into three other families. In contrast, *E. carotovora* produces three major Pels, an intercellular Pel, and several minor plant-induced Pels.

The expression of *Erwinia* genes encoding pectic enzymes and isozymes is sequential. This suggests that the genes are regulated separately. In addition, there are global regulatory systems, like the quorum-sensing system, so as to maximize the activity of the main enzymes. Because of the large number of pectinases involved, disruption of the gene encoding any one of the enzymes is not sufficient to stop cell maceration. Maceration symptoms develop when a soft rot erwinia population reaches a cell density-dependent regulatory, or quorum-sensing, system for extracellular enzymes. Enzyme production is switched on when both numbers of bacteria and the bacteria-secreted inducer homoserine lactose (HSL) have reached a critical level. Disruption of the HSL gene or addition of a gene encoding an enzyme that breaks down HSL leads to the production of mutants with reduced pathogenicity. Presumably, quorum sensing allows the bacteria to multiply within host tissue without triggering host resistance responses, such as the production of phytoalexins. In general, cell wall-degrading enzymes are considered to play a role in pathogenesis by facilitating penetration and tissue colonization, but they are also virulence determinants responsible for symptom development once growth of the bacteria has been initiated.

Some Xanthomonads, e.g., *Xanthomonas campestris* pv. *campestris*, the cause of black rot of crucifers, have genes for two pectin esterases and polygalacturonases, four pectate lyases, five xylanases, and nine cellulases. *X. citri* has no pectin esterases, one less pectate lyase, and three fewer cellulases. Because pectin esterases are important in tissue maceration, their absence in the citrus canker bacterium and presence in the crucifer rot bacterium may explain the symptoms of the two diseases. Other poor pectinolytic bacteria include *A. tumefaciens*, which has only four genes encoding pectinases of any type, and *Xylella*, which has only one gene coding for a polygalacturonase.

Bacterial Toxins as Pathogenicity Factors

Toxins have been known for a long time to play a central role in parasitism and pathogenesis of plants by several plant pathogenic bacteria. *Pseudomonas syringae, P. syringae* pv. *tomato,* and *P. syringae* pv. *maculicola* are primarily associated with production of the phytotoxin coronatine. Coronatine functions primarily by suppressing the induction of defense-related genes, but, as happens with most bacterial phytotoxins, it does not seem to be essential for pathogenicity by all strains.

The bacterium *P. syringae*, along with its pathovars, produces several pathotoxins, including syringomycin.

Albicidins, produced by *Xanthomonas albilineans*, block the replication of prokaryotic DNA and the development of plastids, thereby causing chlorosis in emerging leaves. Albicidins interfere with host defense mechanisms and thereby the bacteria gain systemic invasion of the host plant.

Extracellular Polysaccharides as Pathogenicity Factors

Extracellular polysaccharides (EPS) play an important role in pathogenesis of many bacteria by both direct intervention with host cells and by providing resistance to oxidative stress. In the bacterial wilt of solanaceous crops caused by *Ralstonia solanacearum*, EPS1 is the main virulence factor of the disease. EPS1 is a polymer composed of a trimeric repeat unit consisting of *N*-acetyl galactosamine, deoxy-L-galacturonic acid, and trideoxy-D-glucose. At least 12 genes are involved in EPS1 biosynthesis. EPS1 is produced by the bacterium in massive amounts and makes up more than 90% of the total polysaccharide. EPS likely causes wilt by occluding the xylem vessels and by causing them to rupture from the high osmotic pressure. The main component of EPS in the fire blight bacterium *Erwinia amylovora* is amylovoran, which is biosynthesized and regulated by several clusters of genes. Disturbance of production of amylovoran eliminates pathogenicity in the mutant.

Bacterial Regulatory Systems and Networks

Some plant pathogenic bacteria, such as *R. solanacearum*, the cause of wilt and soft rot diseases of solanaceous and other crops, as well as a successful soil inhabitant, have developed specialized systems of complex regulatory cascades and networks. These systems sense the different environments in which bacteria find themselves and trigger dramatic changes in their physiology by global shifts in gene expression linked to the primary network that fine-tunes virulence and pathogenicity gene expression. The majority of the network components are transcriptional regulators that consist of a transmembrane sensor kinase protein. The protein binds a specific signal molecule and, in response, its kinase transfers a phosphate group from ATP to its partner response regulator in the cytoplasm. This activates the response regulator, which turns on transcription of its targets.

Virulence and pathogenicity genes of *R. solanacearum* are regulated by a complex network of which the core is the phenotype conversion (Phc) system. The system consists of gene PhcA, a lysine-rich type transcriptional regulator, and the products of the operon phcBRSQ, which control levels of active PhcA depending on cell density or crowding. Cells that contain high levels of active PhcA produce large amounts of major virulence factors, such as EPS1 and some exoenzymes, and are very virulent. When PhcA is inactivated, the bacterial cells become quite avirulent and produce almost no EPS1 and exoproteins; instead they activate genes that produce polygalacturonase, siderophores, the Hrp secretion apparatus, and swimming motility. So the PhcA gene acts as a switch mechanism that sometimes promotes the expression of one set of genes while repressing another set, and other times does the opposite. The levels of PhcA in bacterial cells are controlled by the level of 3-OH palmitic acid methyl ester reached in the cells in response to cell density or confinement. The more dispersed the cells, the lower the concentration of 3-OH PAME in the cells, the less the activation of PhcA, and the more the activation of genes for siderophores, swimming motility, etc. When the cells are confined and dense in plant tissues, the concentration of 3-OH PAME builds up, PhcA activation increases, and genes coding pathogenicity and virulence factors (PES I, cell wall-degrading enzymes) are also activated. How 3-OH PAME activates PhcA is not yet known.

Sensing Plant Signaling Components

Agrobacterium tumefaciens has a two-component regulatory system that senses and reacts to the presence of susceptible cells. The system components are a membrane sensor protein, VirA, and a cytoplasmic response regulator protein, VirG. The two components react to exudates of wounded plant cells and initiate transcriptional activation of the *vir* genes. Expression of *vir* genes follows activation of the VirA transmembrane sensor protein by exuding phenolics such as lignin and flavonoids, and especially the phenolic acetosyringone. A number of gene groups are involved in further steps of infection. Mutants lacking these genes totally or greatly lose pathogenicity.

Other Bacterial Factors Related to Pathogenicity

Several other components of the bacterial cell or released by the bacteria appear to play roles as pathogenicity factors. Lipopolysaccharide (LPS) components of the outer cell wall of gram-negative bacteria play a role in the pathogenicity of erwinias. Proof of this is provided by the activation of pathogenesis-related proteins, such as glucanases (Fig. 4-13) in infected plants, and the fact that disruption of the LPS gene in the bacteria reduces their virulence and that protein–LPS complexes from bacteria inhibit the hypersensitive response (HR).

Catechol and hydroxamate siderophores appear to be virulence determinants for erwinias. In the fire blight bacterium *E. amylovora*, its siderophore protects the bacteria by interacting with H_2O_2 and inhibiting the generation of toxic oxygen species.

The peptide methionine sulfoxide reductase, which protects and repairs bacterial proteins against active oxygen damage, is essential for the expression of full virulence of the bacteria.

hrp genes and *avr* genes are associated with the expression of pathogenicity and host specificity and they exist in clusters. *hrp* genes encode proteins called harpins or pilins and are used to make a type III protein secretion system that is used to deliver Avr proteins across the walls and plasma membrane of living plant cells. Avr proteins and, to a lesser extent, harpins induce rapid cell death, which leads to HR; as a result, the infection by the bacteria in incompatible interactions fails. Avr proteins seem to also play a role in compatible host/bacteria interactions. *avr* genes usually determine host specificity at the pathovar and the species level. The role of *hrp* genes in the pathogenesis of soft-rotting erwinias is debatable.

Pathogenicity Genes in Plant Viruses

Viruses have a limited number of genes, but by utilizing the same genetic material in more than one way, viruses are very capable pathogens. All viruses have genes that encode one or more coat proteins that protect its nucleic acid, one or more nucleic acid replicases that produce innumerable copies of its genome, and one or more movement proteins that help the movement of the virus from cell to cell and long distance through the phloem. Several viruses have additional genes involved in virus transmission by vectors or in other ways, production of cellular inclusions, etc. Although all of these proteins are coded by the virus but are produced by the host plant, viruses also utilize host proteins for the essential functions of transcription and movement.

Viral Pathogenicity Functions Associated with the Coat Protein (CP)

Coat proteins of various viruses function in practically every aspect of viral multiplication and dissemination.

Virus Disassembly. Virus disassembly is essential for virus multiplication and the coat protein plays a central role in it. Destabilization of the weaker 5′ end CP RNA releases a few CP subunits, allowing ribosomes to bind to the exposed 5′ end of the RNA and initiate translation of the RNA replicase(s). Active translation provides the force needed to remove the CP subunits. The RNA replicase then interacts with the 3′ end of the RNA to initiate the (–) RNA strand, thereby uncoating the rest of the virus.

Virus Assembly. Virion assembly initiates at the RNA origin of assembly and proceeds in both directions of the RNA.

Virus Movement. Coat proteins apparently interact directly with movement proteins (MP). Some viruses require CP for long distance but not for cell-to-cell movement of the virus. Mutations to the CP in even a single specific amino acid inhibit the systemic infection of host plants. Other viruses absolutely require CP for even cell-to-cell movement, whereas the movement of still other viruses seems to be unaffected by the absence of CP.

Viral Genome Activation. Virus RNAs within the genera *Alfamovirus* and *Ilarvirus* require that unless a few molecules of CP are present, they cannot cause infection on their hosts. CP is probably necessary for the replication of negative-sense RNA viruses.

Symptoms. CPs can modify the symptoms caused by viruses in plants. Minor modifications of the genes of plant viruses, including the CP gene, can result in significant changes in symptomatology. In some cases, changes in a single amino acid result in dramatic changes in symptoms, ranging from stopping host development to death of the host.

Elicitor of Defense Responses. An important aspect of disease induction by a virus is the ability of the virus to neutralize or overcome the defense responses of the host. The resistance of plants to disease is via the hypersensitive response, which blocks further spread of the virus by programmed death of the infected and adjacent plant cells. Plant viral CPs generally act as elicitors of the plant defense response.

CP-Mediated Resistance in Transgenic Plants. Translatable or nontranslatable portions of CP gene sequences used to make transgenic plants confer resistance to the plant to subsequent challenge inoculation with the same or other viruses.

Viral Pathogenicity Genes

It can be concluded from the aforementioned discussion that the coat protein gene of most viruses plays one or many important pathogenicity roles for the virus. There are not enough genes in the genome of any virus to have separate genes for each of its various necessary functions that provide for its survival, multiplication, and spread. The gene encoding the nucleic acid replicase of the virus is obviously essential because without it there would be no virus. The movement protein-encoding gene is a virulence/pathogenicity gene because it enhances the multiplication and spread of the virus to other cells and plants. The same can be said for the gene(s) that encode proteins that make it possible for the virus to be acquired and then transmitted to other plants by one of the vector insects, nematodes, fungi, and so on.

Nematode Pathogenicity Genes

Nematodes attack plants by penetrating mostly root cells through their stylet. They secrete saliva that liquefies the cell contents that they absorb and move on. They enter the roots and move about in them, or they anchor themselves onto some root cells that become specialized and serve as feeder cells for the nematodes. Nematode secretions have been suspected to contain substances that nematodes use to attack their host plants and bring about a successful infection. These substances are presumably involved in hatching, in self-defense, in movement through plant tissue, and in the establishment and maintenance of a feeding site. Nematode secretions derive from several body structures, including the cuticle, amphids, and esophageal gland cells.

Cuticle Secretions

The surface of the cuticle of the infective juvenile is covered with a protein that binds to retinol and the linolenic and linoleic fatty acids, and inhibits the modification of these compounds by lipoxygenases. Peroxidation of linolenic acid by lipoxygenases is one of the steps in the synthesis of jasmonic acid, which is a signal transducer of systemic plant defenses. Also, peroxidation of lipids by lipoxygenases leads to the generation of reactive oxygen species in plants. Therefore, the protein secreted at the nematode cuticle, by inhibiting the lipoxygenase activities, downregulates and protects the nematode from the defense responses by the plant. The production of reactive oxygen species would also be a hostile environment for the nematodes, as are peroxiredoxins, which are a family of peroxidases that

remove hydrogen peroxide produced at the nematode/plant interface. Superoxide dismutase, a scavenger of free oxygen radicals, is also produced in cuticle secretions.

Amphid Secretions

The role of amphids and their secetions in development of disease is not yet clear but all indications are that they play a major role in feeding site formation and maintenance. Two genes encoding two small proteins have been cloned from the amphids, but the role of the proteins in disease development is still not known.

Esophageal Gland Secretions

The esophageal glands in nematodes have for years been recognized as a major source of proteins that play a role in the parasitism of the nematode. Two sequences, one homologous to a hymenopteran venom allergen and the other homologous to a cellulose-binding cellulase-like protein, have been identified. Numerous other genes have been identified and their proteins are being studied.

Although more than 25 major resistance genes (R genes) against nematodes have been found in plants, no products encoded by nematode avirulence genes have been isolated. Of course, not all resistance to nematodes is provided by R genes.

Genetics of Resistance through the Hypersensitive Response

As mentioned previously, the hypersensitive response is a localized self-induced cell death at the site of infection of a host plant by a race or strain of a pathogen that cannot develop extensively in this particular resistant plant cultivar. Thus, the plant species as a whole may be a host to the pathogen species, but individual cultivars (varieties) of the plant may be hosts (susceptible) or nonhosts (resistant) to a particular race or strain of the pathogen. Resistance through the hypersensitive response has been shown to be the result of gene-for-gene systems in which an avirulence (*avr*) gene in the pathogen corresponds to a resistance (R) gene in the host plant. Such gene-for-gene systems that provide resistance through the hypersensitive response occur in diseases caused by obligate intracellular pathogens, such as viruses and mollicutes, as well as in diseases caused by obligate and facultative pathogens, such as bacteria, fungi, and nematodes. Whatever the type of pathogen, it is believed that resistance through the hypersensitive response is the result of recognition by the plant of specific signal molecules, the **elicitors**, produced by the avirulence genes of the pathogen and recognized by R gene-coded specific receptor molecules in the plant. Such recognition causes the activation of a cascade of host genes, which result in a burst of oxidative reactions, disruption of cell membranes, and release of phenolic and other toxic compounds, which then lead to the hypersensitive response, programmed cell death, inhibition of pathogen growth, and thereby resistance (Fig. 4-13). It also leads to the activation of numerous other defense-related genes that result in other types of resistance, including horizontal resistance and systemic acquired resistance.

Pathogen-Derived Elicitors of Defense Responses in Plants

Pathogen-produced elicitors that trigger defense responses in plants include a wide variety of molecules that seem to have little in common. Some elicitors are host specific, i.e., they induce defense responses leading to disease resistance only in specific host varieties, as is the case with elicitors produced by *avr* genes interacting with a matching R resistance gene in a host plant. Most elicitors are general or limited specificity elicitors in that they signal the presence of a potential pathogen to both host and nonhost plants, although some general elicitors are recognized by a small number of plants (Table 4-5).

In nature, the elicitor molecule either reacts directly with the receptor protein encoded by the resistance gene R, or releases compounds or reacts with another host protein (endogenous elicitors), which then interacts with the R-coded receptor.

Avirulence (*avr*) Genes: One of the Elicitors of Plant Defense Responses

Avirulence (*avr*) genes, first identified by H. H. Flor in the 1950s, were only rather recently isolated from bacteria (1984) and fungi (1991), but since then numerous bacterial and fungal *avr* genes have been identified. The *avr* genes make a pathogen avirulent, that is unable to induce disease on a specific variety of the host plant because their protein product warns the plant of the presence and impending attack by the pathogen and the host plant then mobilizes its defenses and blocks infection by the pathogen. In this way, *avr* genes, by warning the host and thereby inhibiting infection by the pathogen, determine the host range of the pathogen at the species and at the race-variety level.

As the gene-for-gene concept implies, in the majority of cases a matching dominant resistance gene (R) in the

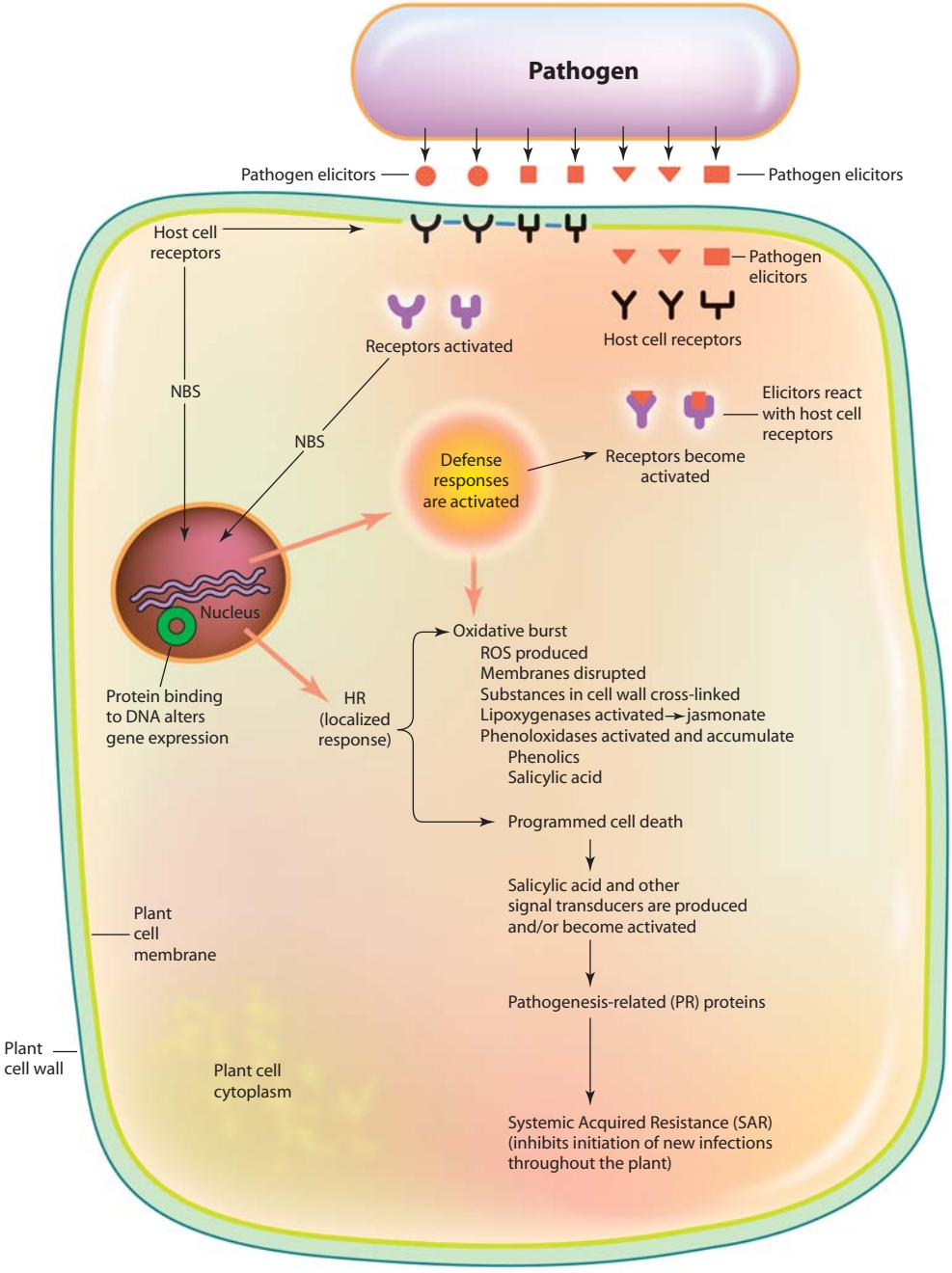

FIGURE 4-13 Basic events in an incompatible host–pathogen interaction: Elicitors from pathogen interact with plant cell receptors. Signal transductions activate hypersensitive (host defense) responses that lead to programmed cell death and systemic acquired resistance.

resistant host corresponds to each avirulence gene in the pathogen. In some cases, however, because two independent resistance (R) genes may correspond to a single *avr* gene, there apparently are genes-for-gene interactions as well. Some *avr* genes, when transferred artificially to other pathovars, are active in the new pathovars, making the recipient pathogen unable to infect their previously susceptible hosts and, instead, causing the hypersensitive response in these plants. In some host–pathogen systems, *avr* genes determine not only which cultivars of a species the pathogen can attack, but also which plant species it can attack. For example, an *avr* gene (*avrBsT*) in the tomato-infecting group of strains of the bacterium *Xanthomonas campestris* pv. *vesicatoria*, the pathogen of bacterial spot in tomato and pepper, enables the bacterium to induce

TABLE 4-5

General elicitors
Glucans, produced by *Phytophthora and Pythium*, derived from oomycete cell wall, induce phytoalexins
Chitin oligomers, by higher fungi, from chitin of fungal cell wall, induce phytoalexins and lignification
Pectin oligomers, by fungi and bacteria, from degraded cell wall, inhibit proteins and defense genes
Harpins, by several gram-negative bacteria, part of type III secretion, cause HR and defense gene response
Flagellin, by gram-negative bacteria, part of flagellum, cause callose formation and defense gene response
Glycoproteins, by *Phytophthora*, induce phytoalexin production and defense gene response
Glycopeptide fragments, by yeast, activate defense genes and ethylene production
Ergosterol, by various fungi, the main sterol of higher fungi, causes alkalinization in cell cultures
Bacterial toxins, such as coronatine of *P. syringae*, toxin, disturbs salicylic acid, mimics jasmonic acid, and induces defense genes and defense compounds
Sphinganine, the fumonisin analog, by *F. moniliforme*, toxin in necrotrophs, disturbs sphingolipid use, induces defense genes and programmed cell death (PCD)
Race-specific elicitors
avr gene products, Avr proteins, by fungi and bacteria, in some cases promoting virulence, HR, and PCD
Elicitins, by P*hytophthora and Pythium,* scavengers of sterol, induce HR in tobacco
Enzymes, e.g., endoxylanase, by *Trichoderma viride*, fungal enzymes, induce defense genes and HR
Viral proteins, e.g., viral coat proteins, by TMV, structural component, HR in tobacco, tomato
Protein or peptide toxins, e.g., victorin, by *Cochliobolus victoria*e, toxin for host, induces PCD in oat
Syringolids (acyl glycosides), by *P. syringae pv. syringae*, signal compound for bacterium, HR in soybean, carrying the Rpg4 resistance gene

the hypersensitive response on all cultivars of pepper. Loss of *avrBsT* from such tomato-infecting strains allows these strains to cause disease on normally resistant pepper cultivars.

Several avirulence genes and the proteins they code have been identified in and isolated from plant pathogenic fungi. These include especially the genes *avr2, avr4, and avr9* of strains of the fungus *Cladosporium fulvum* that are avirulent on tomato varieties carrying, respectively, the resistance loci Cf-2, Cf4, and Cf-9; and the gene *avr*Pi-ta of the rice blast fungus, *Magnaporthe grisea,* which confers avirulence to rice varieties containing the resistance gene Pi-ta. Similarly, several viral *avr* genes and their avr proteins have been obtained and studied, including those of the coat protein of *potato virus X (PVX)*, the coat protein of *turnip crinkle virus (TCV)*, and the replicase protein of *tobacco mosaic virus (TMV)*.

Characteristics of avr *Gene-Coded Proteins*

The gene-for-gene model stipulates that for every dominant gene determining resistance in the host plant, there is a matching dominant gene in the pathogen that conditions avirulence. The biochemical basis for explaining the gene-for-gene concept is the elicitor–receptor model according to which an avirulence (*Avr*) gene of a pathogen encodes an elicitor (Avr) protein that is recognized by a receptor protein encoded by the matching resistance (R) gene of the host plant.

The simplest way of recognition would be if the pathogen-produced elicitor interacted with the protein encoded by the matching resistance gene of the host. Recognition of the elicitor protein by the host plant leads to activation of a cascade of defense responses, which often include cell death around the infection site. The death of cells around the point of infection is known as the hypersensitive response and is characteristic of gene-for-gene-based resistance.

Unlike R proteins, Avr proteins encoded by pathogen *Avr* genes share few common characteristics. Because most *Avr* genes continue to exist within a pathogen population, it would seem that in addition to acting as avirulence factors, *Avr* genes probably have some additional function that is beneficial to the pathogen. From the few *Avr* genes for which a clear function for the pathogen has been demonstrated, it has now become generally accepted that their proteins carry out two functions, one of them being a contribution toward the virulence of the pathogen. Such a contribution appears to come about by the Avr proteins interacting with specific plant proteins, known as virulence targets, involved, for example, in host metabolism or in plant defense. Interaction of Avr proteins with such targets could enhance the availability of nutrients for the pathogen or could suppress defense responses by the host plant. To date, the AvrD protein, produced by the *AvrD* gene of the bacterial spot of tomato pathogen *P. syringae* pv. *tomato,* is the only Avr protein for which a biochemical function has been clearly defined. This function is the ability of the AvrD protein to direct the synthesis of low molecular weight syringolide elicitors, which elicit the hypersensitive response on soybean. A syringolide-binding protein has been identified in resistant soybean plants, possibly representing the protein of the matching R gene of the host plant.

Proteins coded by pathogen *avr* genes (Avr proteins) seem to have some features in common. Avr proteins seem to be generally hydrophilic and, therefore, water soluble, lacking stretches of hydrophobic amino acids that would enable them to be anchored in cell membranes. Avr proteins also lack stretches of amino acids known as "signal sequences" that would allow the

proteins to be secreted into the external medium by the general secretory pathway. It appears, therefore, that *avr* gene proteins are produced and are either localized in the pathogen cytoplasm or they are secreted through membrane pores formed by proteins coded for by hypersensitive response and pathogenicity (*hrp*) genes, known as Hrp proteins (harpins). If they are secreted externally, the Avr proteins may act directly as elicitors. If they are localized in the pathogen cytoplasm, the *avr* gene proteins may act enzymatically to produce an elicitor molecule that is transported freely through the bacterial envelope. In either case, the elicitor reacts directly or indirectly with the product of the corresponding plant resistance R gene (Figs. 4-10 and 4-11).

Structure of avr *Gene Proteins*

Although *avr* genes are quite different, some of them have common structural characteristics that allow grouping of *avr* genes into distinct families. The best known *avr* gene group is the *Xanthomonas avr* gene family, called *pth* (for pathogenicity) genes by some. Members of this gene family are found in different species and pathovars of the bacterium *Xanthomonas*. They encode proteins that, in their central part, have from 13 to 23 copies of a nearly identical 34 amino acid repeat unit. *avr/pth* genes cause the hypersensitive response and are also required for the induction of angular leaf spot symptoms of cotton and for citrus canker disease. Elicitation of these very different symptoms (leaf spots, cankers, the HR) is determined by a single or a few amino acid differences in the repetitive regions of these genes.

Among fungal avr proteins, the *Cladosporium fulvum*-encoded avr2 is a cysteine-rich protein of 78 amino acids that has a signal peptide of 20 amino acids for external targeting; the *Cf* avr4 protein consists at first of a 135 amino acid preprotein, which upon secretion is processed at both ends, resulting in an 86 amino acid protein; and the *Cf* avr9 protein at first consisting of a precursor protein of 63 amino acids, which is further processed into a 28 amino acid peptide. All three *Cf* avr proteins are secreted in the apoplastic space of tomato leaves, are localized in the plasma membrane, and contain an extracellular leucine-rich region (LRR), a transmembrane domain, and a short cytoplasmic tail. The *Magnaporthe grisea*-encoded avr-Pi-ta protein consists of 223 amino acids but is processed into a 176 amino acid protein that has homology to zinc-dependent metalloproteases. The Pi-ta avr protein is cytoplasmic and contains a nuclear-binding site (NBS) and a leucine-rich carboxyl terminus. The viral avr proteins elicit corresponding plant resistance R genes that encode cytoplasmic proteins. These proteins consist, in the case of PVX and TCV, of either LZ-NBS-LRR domains or, as in TMV, of TIR-NBS-LRR domains (LZ, leucine zipper; TIR, toll interleukin 1 receptor).

Function of avr *Gene Proteins*

So far, the functions of only one *avr* gene, *avrD*, have been determined. The *avrD* gene is present in the bacterium *P. syringae* pv. *tomato*, but ArvD alleles are present in soybean *P. syringae* pv. *glycinea* and other hosts. *avrD* encodes syringolide elicitors, which react with the receptor protein of R gene, Rpg4 of soybean, and confers avirulence on soybean. It has no effect on the virulence of the bacterium.

The function of fungal avr proteins is not known with certainty. The timing and location of their expression suggest a role in the infection process, but so far no virulence function has been reported for most such proteins. In the case of the avrPi-ta protein, direct interaction was detected between the mature avrPi-ta protein and the leucine-rich domain of the Pi-ta R gene protein. This finding is the first experimental evidence consistent with the proposed model that avr proteins interact directly with the corresponding R proteins.

In the case of *tobacco mosaic virus*, causing the hypersensitive response in *Nicotiana sylvestris* tobacco carrying the N^1 gene for resistance, the avirulence function and thereby the elicitation of hypersensitive response seem to reside in the presence of certain amino acids on the coat protein of the virus: N^1 gene-containing plants transformed with only the gene of such TMV elicitor coat proteins, without inoculation with the virus, exhibited the hypersensitive response in the form of reduced growth, chlorotic and necrotic patches, and eventual collapse of entire leaves. Plants transformed with mutant weakly eliciting or nonelicitor coat proteins expressed respectively weaker or no hypersensitive response. In at least some viral infections then, the viral coat protein, which is produced within the cell, appears to function as a specific elicitor that activates the hypersensitive response in plant cultivars that carry the corresponding R gene for that virus.

Role of avr *Genes in Pathogenicity and Virulence*

Most *avr* genes tested so far play no role in pathogenicity or virulence of the pathogen, as even when *avr* genes are inactivated by mutation, susceptible hosts continue to be susceptible. Some *avr* genes, however, e.g., the *avrBs2* gene from the bacterium *X. campestris* pv. *vesicatoria*, encode proteins that are also necessary for pathogenicity. This is shown by the fact that this *avr* gene is present in all strains of this pathovar, whereas

mutants lacking the *avr* gene lose pathogenicity on all susceptible hosts but do not gain virulence on any previously resistant hosts. However, several *avr* genes, such as the *pthA* gene from *X. citri* and *avrb6* from *X. campestris* pv. *malvacearum*, both members of the *Xanthomonas avr/pth* gene family, encode proteins that act as pathogenicity or virulence factors. For example, they enhance the virulence of a weakly pathogenic leaf-spotting strain of *X. citrumelo*, enabling it to cause canker-like lesions on its host; they may act as pathogenicity factors, e.g., *pthA* is required for the pathogenicity of *X. citri* on citrus to cause the typical citrus canker disease; and act as avirulence genes, e.g., by causing *pthA*-transformed strains of *X. phaseoli* and *X. campestris* pv. *malvacearum* that, respectively, infect bean or cotton, but not citrus, to cause the hypersensitive response on their respective hosts bean and cotton while remaining nonpathogenic on citrus.

The role of fungal *avr* genes in pathogenicity and virulence of the pathogens involved is mostly unclear. In some cases, avr proteins seem to react with other proteins that play an intermediate role in transmitting the signals for plant defense. In a few cases, as in the avr Pita protein, they seem to interact directly with the R protein and to set off a cascade of defense reactions. In viruses, a certain segment of a particular coat or replicase protein seems to interact with the host R gene. Most of these statements, however, need further experimentation to support their validity.

hrp *Genes and the Type III Secretion System: Another Class of Pathogenicity Genes in Bacteria*

The *hrp* (hypersensitive response and pathogenicity) genes, found only in gram-negative bacteria so far, are additional bacterial genes that seem to be essential for some bacteria to be able to cause visible disease on a host plant, to induce a hypersensitive response on certain plants that are normally not infected by the bacteria, and to enable bacteria to multiply and reach high numbers in a susceptible host. Most bacterial species have two distinct clusters of *hrp* genes. The larger *hrp* gene cluster consists of six to nine transcription units, with each transcription unit coding for several (1 to 12) proteins. The transcription of *hrp* genes is controlled by the presence of certain nutrients, by other bacterial regulatory genes, and by so far unknown signal molecules of plant origin.

Several *hrp* gene-coded proteins, called harpins, seem to be localized in the bacterial cell membrane (Fig. 4-11). There they may be involved in forming a type III secretory apparatus involved in the outward translocation of bacterial Avr or Hrp proteins that could interact with components of host plant cells. Some *hrp* genes also code for an ATPase enzyme that may play a role in energizing the secretory apparatus.

In some bacteria, e.g., in *P. syringae*, a single promoter gene controls the expression of both *hrp* and *avr* genes, including the production of a harpin, a secretion system for harpins, and the *avr* products that elicit the hypersensitive plant response and affect the host range of the pathogens. The coregulation of both *hrp* and *avr* genes suggests that the final effectors of these genes may act together to determine the final outcome of the plant–bacterium interaction.

Resistance (R) Genes of Plants

As mentioned earlier, despite the many and different kinds of plant pathogens that come in contact with a plant, in most cases, plants remain resistant to disease because they are not hosts to the vast majority of pathogens (nonhost resistance). What makes a plant nonhost to most pathogens and host to a small number of pathogens (usually about 50–100) is still not known. Even when a plant is a host (i.e., is susceptible) to a certain pathogen, some varieties of the plant may be susceptible, or more susceptible, to the pathogen, whereas others may be resistant, or more resistant, to the pathogen. This depends on the kind and number of resistance genes present in the plant, the prevailing environmental conditions, and other factors. Even when a plant becomes attacked and diseased by a pathogen, however, a number of defense response (resistance) genes are activated. As a result, in most cases, the plant manages to limit the spread of the pathogen into a smaller or larger spot, lesion, canker, and so on through defense compounds and structures that block the further expansion of the pathogen. In a number of cases, however, plant varieties are resistant to certain pathogen races because they possess specific resistance (R) genes that enable the plant to remain resistant to pathogens carrying the corresponding avirulence (*avr*) genes.

So far, a number of plant R genes and pathogen *Avr* genes have been cloned and characterized. The proteins encoded by R genes are quite similar and are classified according to certain structural characteristics they have and according to their localization in the plant cell (Fig. 4-14). All R proteins except two contain a domain rich in the amino acid leucine (LRR, leucine rich repeats), which is thought to take part in protein–protein (e.g., elicitor–receptor) interactions. Depending on where in the plant cell the R protein LRR reside, they have either cytoplasmic LRRs or extracytoplasmic LRRs. The R proteins that have a cytoplasmic LRR domain also have a nucleotide-binding site (NBS) and some of them have a zipper-like domain of leucine molecules known as coiled coil, or have a domain of Toll/interleukin 1 recep-

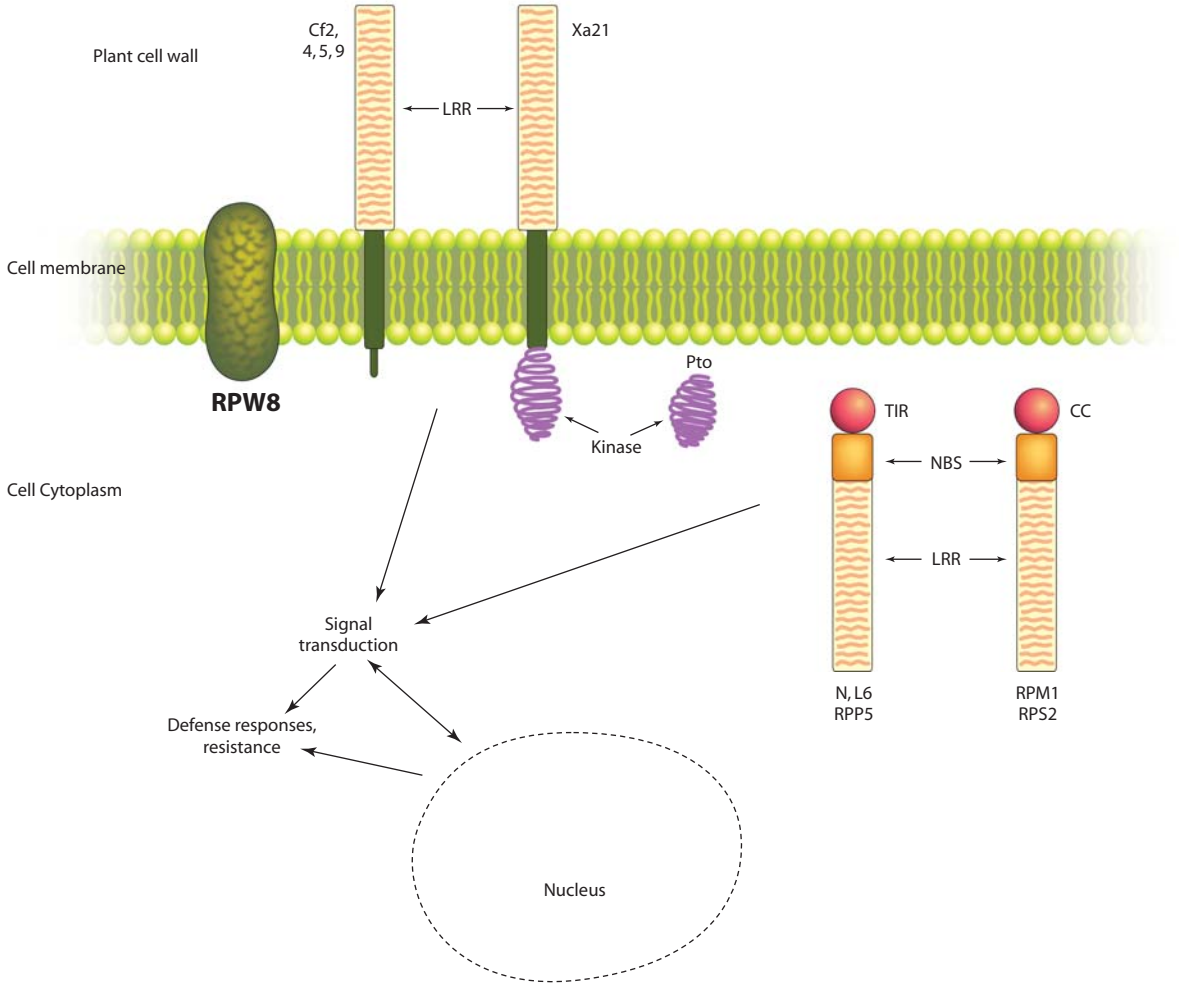

FIGURE 4-14 Schematic diagram of the structure and cell location of the six types of R-coded receptor proteins. Three types have transmembranous domains, while the other three are membrane-associated cytoplasmic proteins. LRR, leucine-rich repeats; NBS, nucleotide-binding site. TIR, Toll–interleukin-1 resistance receptor domain; CC, coiled coil with leucine zipper domain. Genes listed are tomato *Cf*-2, -4, -5, -9, rice Xa21, tomato Pto, tobacco N, flax L6, Arabidopsis RPM1, RPS2, RPP5, and the Arabidopsis broad-spectrum gene RPW8.

tor (TIR). A different kind of R gene named RPW8 has been found in *Arabidopsis*. RPW8 is different in that it confers resistance to a broad range of powdery mildew pathogens instead of a specific pathogen race. The RPW8 protein is located in the plant cell membrane but its mode of action is not known yet. The R proteins that have an extracytoplasmic LRR domain contain a transmembrane region, and some of them also contain a cytoplasmic domain that acts as a protein kinase. Although the structure of R proteins predicts a role for them in signal transduction, it is not clear how these proteins initiate defense responses.

Examples of R Genes

In 1992, the first R gene, the maize *Hm1* gene, was located, isolated, and sequenced, and its function was described at the molecular level. The *Hm1* R gene makes corn plants of certain varieties resistant to race 1 of the fungus *Cochliobolus carbonum*, which causes a leaf spot disease on susceptible corn varieties. Race 1 of *C. carbonum*, the asexual stage of which is *Bipolaris (Helminthosporium) carbonum*, produces a host-specific toxin, the HC toxin. The toxin is a pathogenicity factor for race 1 because the latter must produce HC toxin if it is to infect the corn varieties that lack the *Hm1* gene and are susceptible to the fungus. However, in corn varieties resistant to race 1, expression of the *Hm1* gene results in the production of an enzyme called HC toxin reductase. This enzyme reduces and thereby detoxifies the HC toxin and in that way keeps the plants free from infection by the fungus. If the HC toxin gene of some race 1 isolates is inactivated artificially, these isolates lose the ability to infect corn varieties that do not carry

the *Hm1* gene and, therefore, the genetics of this host–pathogen system are not quite the same as in the typical gene-for-gene systems.

Within 3 years after isolation of the *Hm1* gene, more than a dozen plant R genes that conform to the classic gene-for-gene relationship were isolated from plants, sequenced, and transferred and expressed in other, susceptible, plants. The first such gene was the *Pto* gene of tomato, so called because it confers resistance in tomato to the bacterial speck-causing strains of *P. syringae* pv. *tomato* that carry the avirulence gene *avrPto*. The protein encoded by the *Pto* R gene appears to be a serine–threonine protein kinase, an enzyme suspected to play a role in signal transduction leading to the hypersensitive response. The *Pto* R gene appears to be one of five to seven homologous R genes that exist as a cluster on one of the tomato chromosomes.

Some of the other R genes isolated from plants include the tomato *Cf2*, *Cf4*, *Cf5*, *and Cf9* genes, which confer resistance to the leaf mold-causing fungus *Cladosporium fulvum* races 2, 4, 5, and 9 that carry the avirulence genes *avr2*, *avr4*, *avr5*, and *avr9*, respectively; the tobacco N^1 gene, which confers resistance to TMV; the flax L^6 gene, which confers resistance to the rust fungus *Melampsora lini* race 6 carrying the *avr6* gene; the rice *Xa21* gene, which confers resistance to many races of the leaf-spotting bacterium *Xanthomonas oryzae*; and several *Arabidopsis* R genes (Table 4-6).

How Do R Genes Confer Resistance?

The mechanisms by which R genes bring about disease resistance to a plant against a specific pathogen are not yet understood. It is believed that the elicitor molecule produced by an *avr* gene of the pathogen is recognized by a specific plant receptor encoded by an R gene. What happens next is mostly speculation. Following recognition of the elicitor by the receptor molecule, one or more kinase enzymes may become activated, which then amplify the signal by phosphorylating, and thereby energizing, other kinases and other enzymes. This leads to a cascade of biochemical reactions that, in ways that are still unclear, result in the hypersensitive response and, thereby, localized host resistance at the point of attack by the pathogen. Of course, in many cases, the hypersensitive response is followed by the development of various levels of systemic acquired resistance (SAR), which is expressed in the vicinity of attack as well as in distant parts of the plant.

Evolution of R Genes

It is thought that when a plant was first attacked by a new pathogen strain, the plant probably had some genes encoding nonspecific receptor molecules that enabled the activation of defense responses to wounding and to pathogens in general but that it lacked any R genes to the new pathogen (Fig. 4-15). This pathogen, therefore, was able to cause considerable damage to the plant and possibly killed many of the susceptible plants. Plants exhibiting greater or lesser general resistance survived and multiplied to proportional extents. When, during the evolutionary race for survival of the plant from the pathogen, a resistance (R_1) gene evolved, e.g., by modification of one of the general resistance genes, and that gene allowed the plant to recognize one of the initial steps of infection by the new pathogen (race 1) and to resist infection, such an individual plant and its progeny (variety 1) were selected for survival and so the plant and the R_1 gene survived and multiplied. This might have happened, for example, by modification of one of the receptors involved in activating plant defenses against pathogens in general. Thus, the modified receptor 1 product of the R_1 gene recognizes specifically a particular compound (elicitor 1) produced by a pathogen gene, which gene, as a result, behaves like an avirulence (*avr1*) gene. Pathogens carrying this *avr1* gene (race 1) cannot survive on such R_1 gene-carrying plants. If, however, in time, a mutation affects the *avr1* gene of race 1 of the pathogen, which gene until now was the cause of its avirulence, the gene and the avirulence are destroyed. As a result, the new offspring of the pathogen become virulent again, capable of attacking the so-far resistant variety 1 of the plant. This new virulent pathogen population could be called race 2. The host plant (variety 1) is now susceptible to race 2, which

TABLE 4-6
Classes of Plant R Gene Proteins

Class	Function	Example of R gene
I	Membrane–associated, transcription regulating, mediating broad-spectrum resistance	RPW8
II	Cytoplasmic signal-transducing serine–threonine protein kinase	P*to*
III	Extracellular LRRs with transmembrane anchor	*Cf-2–Cf-9*
IV	Extracellular LRRs, with a transmembrane receptor and a cytoplasmic serine–threonine kinase	Xa21
V	Cytoplasmic, membrane associated. Contain LRRs, NBS, and TIR domains	RPP5, N^1, $L6_{6,RRPP}$
VI	Also cytoplasmic, membrane associated. Contain LRRs, NBS, and a coiled coil domain	RPM1, RPS2

infects and may kill many plants. Soon, however, through survival pressure and selection, an R_2 gene evolves that encodes a new or further modified receptor 2 that recognizes a different compound (elicitor 2) produced by the *avr* gene of individuals of the pathogen race 2. This gene, then, becomes the *avr2* gene conferring avirulence to the pathogen because it is recognized by the R_2 gene of the plant. In this way, numerous, diverse R genes have evolved in a plant host to counteract corresponding virulence genes in the various races of one of its pathogens. This gene-for-gene interaction has occurred in a stepwise fashion over time and continues to date (Fig. 4-15).

The evolutionary process just described is supported by the fact that most of the R genes studied so far seem to be present in tandem arrays of multiple (up to 10 or more) related R genes: They exhibit different specificities but behave as though they are alleles of a single gene that cannot be separated during recombination or exist as a clustered gene family. The various R genes isolated so far appear to have a portion (about 20%) of their nucleotide sequences identical, whereas a larger portion (about 50%) of the nucleotide sequences are similar. Such relationships among R genes may indicate an important mechanism by which plants, by reshuffling preexisting coding information, can respond more quickly to attack by a new pathogen by reformulating existing R genes into new R genes that then produce new specific receptors. The latter are needed, of course, to recognize one of the diverse molecules produced by pathogens, which in any case, because of their extremely large populations, change at a much greater frequency than plants can produce R genes. Besides, the change of a pathogen from avirulence to virulence is caused by the loss of an avirulence gene through a loss of function mutation on that gene, an event much more likely to happen than the positive production of a new receptor on a plant by a newly formed R gene (Fig. 4-16).

Other Plant Genes for Resistance to Disease

As mentioned earlier, how many and what types of genes make a plant nonhost, and therefore resistant, to a pathogen are unknown. It is possible that nonhost resistance is due to a lack of host recognition factors so that the pathogen is not triggered to express its pathogenicity factors on such a plant. Alternatively, it is possible that the nonhost plant carries numerous R gene-coded receptors, one or more of which quickly recognize and fend off the pathogen, or, probably, some entirely different mechanisms are responsible for nonhost resistance.

R genes, as discussed, are responsible for recognition by certain plant varieties of specific elicitors produced by certain pathogen races. That recognition results in the production of signal molecules, some of which trigger a cascade of localized reactions, leading to the hypersensitive response and, through the plant-induced death of the affected cell, to localized resistance. Such spectacular, easily identified R gene-dependent resistance is rather rare in natural genetically heterogeneous plant populations, but has been introduced into culti-

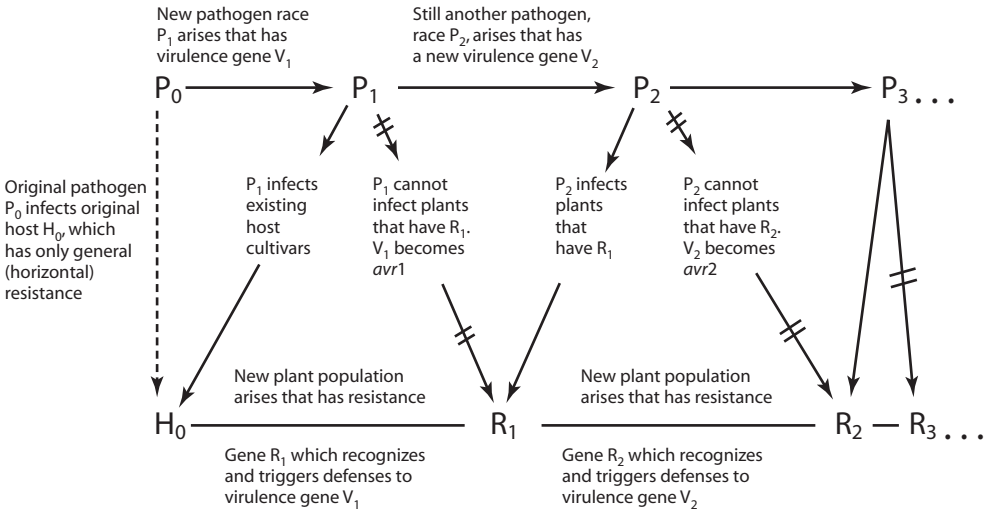

FIGURE 4-15 Steps in the evolution of genes for virulence, resistance, and avirulence. Note that race 1 pathogens (P_1) can still infect hosts carrying only the original resistance or R_2 resistance; they cannot infect plants with R_1 resistance. Also, plants with R_1 resistance are only resistant to P_1 pathogens that carry the V_1 (*avr*1) gene. R_1-carrying plants are susceptible to the original pathogen population (P_0) and to other pathogen races, e.g., to P_2.

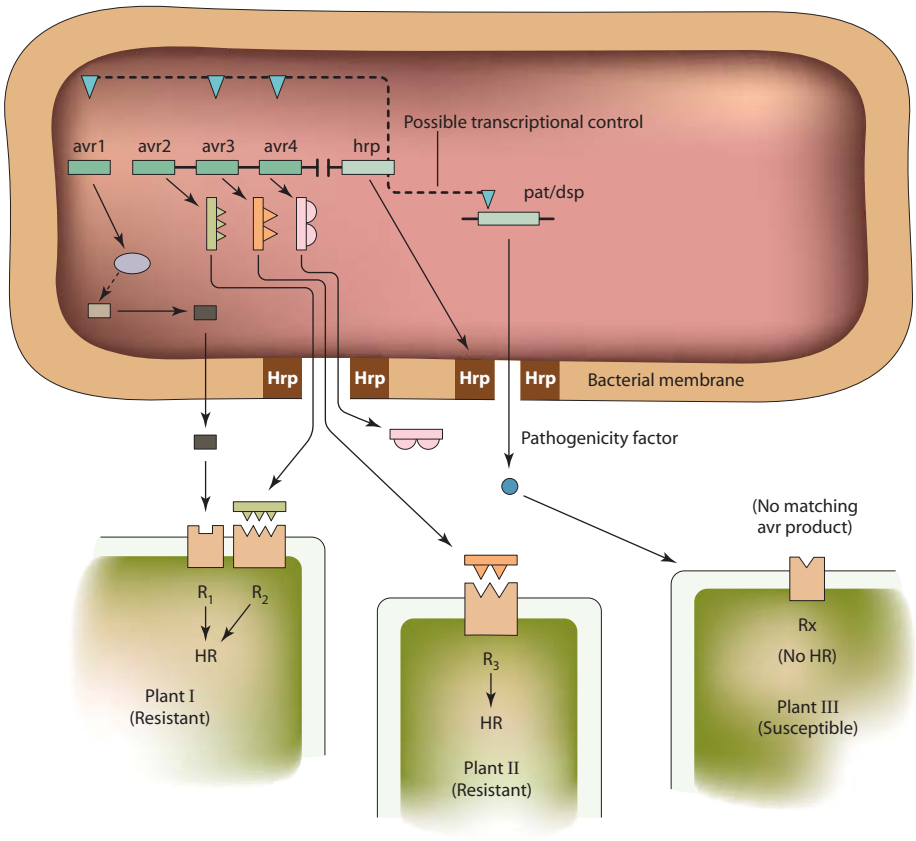

FIGURE 4-16 A simplified scheme of hypothetical molecular interactions between *avr* and *hrp* genes of a pathogenic bacterium and the R genes of two resistant and one susceptible plant. In this diagram, the avr1 product induces an intracellular enzyme to produce an elicitor that moves freely through the bacterial envelope. The products of *avr*2, *avr*3, and *avr*4, as well as effector proteins transmitted through the type III secretion system, move through membrane pores formed by the proteins (harpins) of *hrp* genes and act as elicitor molecules on receptors encoded by corresponding R genes. The pathogenicity/disease specificity (*pat/dsp*) genes are likely producers of effector proteins. From Van Gijsegen *et al.* (1995).

vated crops by breeding and is now quite common in commercial crops.

During development of the hypersensitive response, some of the signal molecules act on other signal molecules that transmit the alarm to other cells and to most distal parts of the plant. There, they trigger the activation of additional defense response genes called systemic acquired resistance genes. These genes mobilize the host defenses throughout the plant and are effective against new infections by the same pathogen and also against infections by unrelated pathogens.

The most common types of resistance genes in plants in natural populations, and quite often in cultivated crops, are numerous "minor" genes for resistance. These may affect superficial or internal, structural or biochemical defenses, preexisting or induced on or after infection. Such minor genes are probably quite numerous in all plants. They are triggered into action by signal compounds produced by the pathogen or by the infected cells and, in most cases, through their actions, produce defenses that manage to halt the advance of the pathogen and colonization of the host to a small lesion on whatever plant organ is attacked. Such minor defense genes do not always appear to effectively defend plants from pathogens, primarily because the pathogens can overcome their hosts by the sheer number of small lesions they cause on the plants. Nevertheless, in most cases, these genes manage to halt the pathogen to a small lesion in each individual infection.

Signal Transduction between Pathogenicity Genes and Resistance Genes

Induced defenses of plants against pathogens are regulated by networks of interconnecting signaling pathways in which the primary components are the plant signal molecules salicylic acid (SA), jasmonic acid (JA), ethylene (ET), and probably nitric oxide (NO). In many

host/pathogen interactions, plants react to attack by pathogens with enhanced production of these substances while a distinct set of gene-to-gene resistance defense-related genes is activated and attempts to block the infection. Also, an exogenous application of SA, JA, ET, or NO to the plant often results in a higher level of resistance.

Salicylic acid reacts with several plant proteins, including the two major H_2O_2-scavenging enzymes catalase and ascorbate peroxidase, and with a chloroplast SA-binding protein, which also has antioxidant activity. The main components of the SA-mediated pathway leading to disease resistance appear to be constitutively expressed genes encoding pathogenesis-related (PR) proteins. Some of these genes also activate the JA- and ET-mediated pathways, leading to induction of the gene encoding defensin. However, NO synthase activity also increases dramatically upon inoculation of resistant but not of susceptible plants. NO induces the expression of PR-1 and the early defense gene phenylalanine lyase (PAL). Production of SA occurs within the NO-mediated pathway downstream of NO. As with SA, NO also reacts with and inhibits the activity of the enzymes aconitase, catalase, and ascorbate peroxidase. SA is not generally required for action of resistance genes R in determining resistance at the infection site, but in at least some plants, SA is required at the primary infection site and in distal secondary tissues for the establishment and maintenance of SAR. Current thinking, however, has HR based on the interplay and mutual positive feedback regulation of reactive oxygen intermediates (ROI) and SA-dependent signals. These, however, may not be the only signals required to set the HR cell death threshold. ROI and NO, generated independently during the oxidative burst, also collaborate to initiate HR. A balance between hydrogen peroxide, derived from dismutation of superoxide, and NO is required for HR. As a result, superoxide is a key regulator that can either convert NO into inert ONOO— or be dismutated to H_2O_2, keeping in mind that superoxide dismutase is induced rapidly by SA.

It is not known how plants integrate signals produced by different defense response pathways into specific defense responses. It is known, however, that the defense pathways dependent on SA, JA, ET, or NO affect each other's signaling either positively or negatively. This is called "cross talk" between pathways. Cross talk provides a regulatory potential for activating several resistance mechanisms in various combinations at once and may play a role in selecting for activation a particular defense pathway over others available. Due to negative cross talk, however, it is often assumed that SA-dependent defenses are often mutually exclusive with JA/ET–dependent defenses.

Signaling and Regulation of Programmed Cell Death

The hypersensitive response, which results in localized, very rapid cell death at the site of attempted pathogen ingress, is found in nearly all defense responses: Those mediated by one or more R genes, by nonhost resistance, and in many cases of polygenic or quantitative resistance. Interaction between elicitor and receptor molecules immediately leads to signal transduction during rapid ion flux. This results in alkalinization of the extracellular apoplast, formation of reactive oxygen intermediates, production of nitric oxide, activation of signaling cascades involving MAP kinase pathways, and transcriptional activation of a broad range of defense genes. This transcriptional reprogramming results in the production or release of antimicrobial compounds, or in the generation of signaling molecules that will act at distal points of the plant to establish systemic acquired resistance.

The extent of cell death during an HR can vary from one cell to tens of cells at the point of infection. Again, not all disease-resistant reactions lead to cell death. Depending on the "efficiency" of the R protein, resistance may be achieved without HR or, if less efficient, an R protein may require more ion flux and thus initiate HR. It should be kept in mind, however, that SA, JA, ET, and NO play a role not only in HR and programmed cell death, but also in decisions about cell growth. This strongly suggests that the relationship between SA and ROI and the genes they regulate is pivotal among signals that determine whether cells live or die. Considering that there are mutants in several species that, with their own plant genes, initiate cell death in the absence of any pathogen, HR can be considered as "programmed cell death." Such mutants could be thought of as representing a step along normal disease-resistant response pathways. Alternatively, they could be thought of as representing changes of normal metabolism that the cell senses and interprets as a commitment to rapid cell death by which it silences the renegade cell forever. Once the plant commits to the cell death pathway, it must be able to stop cell signals that might propagate cell death to neighboring cells, especially since plants have no scavengers that can engulf the corpses of cells killed by programmed cell death.

Some light on the mechanism of programmed cell death has been shed by the discovery of a recessive allele [the lesion simulating disease (lsd1)]. This allele leads to a lowered threshold for signals derived from pathogens, ROI, or SA and entering the disease resistance pathway. Disruption of this gene leads to mutant plants that are unable to stop the spread of cell death once it has started. Local applications of low concentrations of the signal molecule SA, of any of the other chemicals that

activate "systemic acquired resistance," of pathogenic bacteria or fungi, or a shift to nonpermissive long-day conditions initiate foci of dead cells. These quickly become "runaway cell death" (rcd), which spreads beyond the initial site of infection and kills the entire inoculated leaf. Cell death, however, does not spread beyond the treated leaf. It has been shown that lesions form in leaves of treated lsd1-carrying plants as a result of accumulation of extracellular superoxide, to which these cells are extra sensitive, and cell death is initiated. This leads to subsequent superoxide formation in live neighboring cells, which leads to further superoxide formation and spread and to runaway cell death. At least one necrotrophic plant pathogenic fungus, *Botrytis cinerea*, attacks and induces runaway cell death in plants carrying the lsd1 gene. Upon infection, the fungus releases hydrogen peroxide or superoxide, which is converted rapidly by superoxide dismutase into hydrogen peroxide. This activates and stimulates the plant HR pathway. Thus, the fungus, by usurping the HR signaling and programmed cell death, subsequently invades the dying tissue and then continues to colonize the plant by mimicking the HR signals.

Genes and Signaling in Systemic Acquired Resistance

Systemic acquired resistance in plants is a secondary resistance response induced after a hypersensitive response to avirulent pathogens. The signal for SAR may be generated within 4–6 hours from inoculation. SA could be detected in the phloem by 8 hours after inoculation, and increases in SA occurred in the phloem of the leaf above the inoculated one within 12 hours from inoculation of the lower leaf. Expression of SAR occurred within 24 hours from inoculation. By that time the entire plant contained greatly increased levels of SA, even when the inoculated leaf had been removed before any SA increase had been detected. Plants transformed with the nahG gene, which codes for the enzyme salicylate hydroxylase, that breaks down SA to the simple phenolic catechol, cannot accumulate SA and cannot express SAR. Also, plants with suppressed phenylalanine lyase activity, a compound that is a precursor to SA, were more susceptible to infection.

External application of SA on plant tissues induces resistance to disease. At the same time, several suspected defense genes are induced systemically by the SA treatment, just as they are induced by various pathogens.

The finding that catalase binds to SA led to considering catalase as the compound that induces levels of resistance along with tissue necrosis and accumulation of PR-1. It was also shown that both H_2O_2 and SA are in the same signaling pathway, but that SA acts downstream of H_2O_2. More recently, nitric oxide has been shown to be an additional signal for the expression of defense. Application of NO on plants releases agents that induce the accumulation of phytoalexins, whereas inhibition of NO synthesis increases the susceptibility of plants. NO and SA both induce PR-1, but only NO induces PAL and accumulation of SA. These and other observations prove that NO acts through induction of SA.

The establishment of SAR follows production and accumulation of the systemic signal salicylic acid at the primary infection site, and in both local and systemic tissues. This leads to activation of numerous effector genes, the proteins of some of which are known as pathogenesis-related (PR) proteins. Concerted expression of these genes results in broad resistance to diverse pathogens. It should be kept in mind, however, that SAR is just one component of the defense responses and that possibly several defense pathways are essential for the full expression of pathogen-induced resistance.

One of the first steps toward SAR is overexpression of the NIM1/NPR1 gene, the protein of which is essential for transduction of the SA signal. This protein is translocated to the nucleus, where, in the presence of SA, nuclear localization of the genes results in regulated expression. How this gene regulates expression of other genes is not known, but it has been shown that its protein contains two domains that are involved in protein–protein interactions. The same gene has been found to interact with a subfamily of transcription factors that have been implicated in regulating SA-mediated gene expression.

Examples of Molecular Genetics of Selected Plant Diseases

The Powdery Mildew Disease

Powdery mildew fungi are obligate plant pathogens that attack approximately 10,000 species of plants belonging to more than 1600 genera. As obligate biotrophs, powdery mildew fungi obtain their nutrients from living cells of their host plants through specialized feeding organs, the haustoria. Powdery mildews evolved effective secretive ways of feeding and pathogenesis, effective counterdefense mechanisms that neutralize the host's defenses, or effective pathways for scrambling defense signaling. Numerous pathogen and host genes become involved in each of the steps in a successful infection, including recognition of host and pathogen, adhesion of fungal spores to host surfaces, spore germination, appressorial initiation and development, penetration peg development, peg penetration into host cell, haustorial initiation and development, neutralization

of host defenses, removal of nutrients from host cell, hyphal growth, and sporulation (Fig. 4-12).

Halting of powdery mildew attacks by the host can be accomplished by single dominant loci of varying strength, such as R resistance genes; by single host genes that mutated to a recessive loss of function, such as barley Mlo and the Arabidopsis EDR1 and PMR1-PMR4 genes; or by the combined, additive effects from many genes.

The barley Mla locus is a race-specific R locus that confers resistance to at least 32 *Blumeria graminis* f. sp. *hordei* (Bgh) resistance specificities. Mla occupies a 240-kb chromosome section adjacent to eight nucleotide-binding, leucine-rich repeat (NB_LRR) type R gene homologs. Several other groups (MLA1-MLA12) of resistance specificities have been found with genes encoding coiled coil (CC)-NB-LRR type R proteins more than 90% identical and having four common introns. Some of the gene specificity domains have different but overlapping regions in the LRR domain that determine avirulenve (*Avr)* gene specificity. Three *avr* genes were found to be linked and to be located at an interval of about 8 kb.

Another barley gene, Rar1, was found to be needed for the resistance triggered by a subset of R specificities encoded by Mla, and also for powdery mildew R genes located on other chromosomes. Similarly, disruption of the Arabidopsis homolog, AtRAR1, produced mutant plants that had no resistance conferred by R genes against the downy mildew oomycete *Peronospora parasitica* or the bacterium *Pseudomonas syringae*. Furthermore, gene silencing of the RAR1 homolog of *Nicotiana benthamiana* destroyed the function of the tobacco N gene against *tobacco mosaic virus*. This points out the conserved function of RAR1 in resistance to diseases caused by pathogens of different taxonomic groups and in plants of different families. Actually, not only is RAR1 highly conserved in many plant species, homologs of it are also found in other eukaryotic organisms, including animals. Another highly conserved eukaryotic protein is SGT1. R proteins have varying requirements for RAR1/SGT1 that range from a total dependency on RAR1 or STG1 to dependency on both proteins to complete independence from both.

A resistance locus RPW8 is unusual in that it confers dominant resistance to several different powdery mildew species. Two RPW8 clustered sequence-related genes encode novel proteins about 17–20 kDa. These have several hydrophobic stretches found in transmembrane domains and are each able to provide resistance against several powdery mildews. Cellular events observed in PRW8-mediated resistance are similar to those in race-specific resistance, including an oxidative burst, a HR-like programmed cell death, and induction of PR-1 proteins. The similarities even include sharing regulatory components such as RAR1, and a requirement for the accumulation of salicylic acid. Depletion of SA reduced partially the race-specific resistance. Mutants of gene EDS1, which presumably encodes a lipase, and of NDR1, eliminate preferentially the race-specific resistance triggered by the intracellular R proteins composed of TIR-NB-LRR and CC-NB-LRR. It is proposed that RPW8 proteins act as compatibility factors that make possible the delivery of one or several powdery mildew pathogenicity factors into host cells. RPW8 leads to the exposure of conserved pathogen-associated molecular patterns that are recognized by acceptors of pattern recognition.

Barley plant mutants with homozygous alleles (mlo) of the Mlo gene are resistant to all tested isolates of Bgh and show increased susceptibility to the rice blast fungus *Magnaporthe grisea*. It should be noted that the mutants do not express defense responses constitutively, as proven by the absence of expression of PR genes in non-challenged plants. During leaf senescence, however, they cause the leaves to develop spontaneous spots of dead mesophyll cells and accelerated pigment removal. Mlo, therefore, appears to change defense responses to Bgh and to *M. grisea* in opposite directions and to negatively regulate certain events during leaf senescence.

A special feature of resistance of mlo to Bgh is that fungal pathogenesis stops at the time the penetration process through the cell wall is complete and does not lead to a hypersensitive response. This typically happens in most R gene-triggered responses. Instead, attempts by the fungus to enter plant cells of susceptible (Mlo) and resistant (mlo) plants cause the cells to remodel their cell wall beneath the fungal appressoria and to produce ring-shaped cell wall appositions. Although some feel that cell wall appositions serve as a scaffold and facilitate fungal pathogenesis, they most likely lead to structural reinforcement of the cell wall. In addition, cell wall appositions are resistant to cell wall-degrading enzymes and are sites of accumulation of hydrogen peroxide and other reactive oxygen species, as well as several phenolic compounds.

The MLO protein is an unusual transmembrane protein, about 60 kDa, and has seven transmembrane helices. It was the first of a family of proteins unique to plants with more than a dozen members each in rice and Arabidopsis. It shares common properties with animal and yeast G proteins, but MLO defense modulation to Bgh functions independently from G-protein silencing of single cell genes.

Magnaporthe grisea, *the Cause of Rice Blast*

Rice blast is one of the most severe diseases of rice. *M. grisea* has seven chromosomes and a genome size of

40 Mb, with approximately 9,000 genes. The pathogen is a haploid ascomysete that produces conidia on aerial conidiophores emerging from the center of lesions. The conidia consist of three cells. Each conidium contains an adhesive glycoprotein that, when wet, sticks tightly to the leaf surface. The conidium germinates rapidly from one of the terminal cells and attempts to penetrate the leaf surface. Within about 4 hours, the apex of the germ tube becomes swollen and flattened, the nucleus divides mitotically, and one daughter nucleus migrates into the appressorium being formed at the leaf surface. The appressorium differentiates by having thickened cell walls and a layer of melanin laid in the appressorium cell wall. These structural additions and the presence of glycerol increase the turgor pressure of the appressorium so that the penetration peg produced at the bottom of the flattened appressorium penetrates the cuticle and the cell wall and enters the cell. New disease lesions become apparent about 4 days after inoculation.

Numerous genes encoding proteins that act during contact and adhesion of the spore with the host and during appressorium formation have been identified. A hydrophobin protein (MPG1) produced in large amounts during appressorium formation helps appressoria to recognize hydrophobic surfaces. Disruption of the gene reduces appressorial formation on hydrophobic surfaces. The addition of cAMP to such mutants overcomes its handicap, indicating an efficient transmission of a surface signal so that appressoria can form via cAMP. A possible mechanism of transmission of the signal is through receptor PTH11, a possible membrane protein that has been identified. Mutants missing *Pth11* do not form appressoria and cannot infect plants. Another gene, mag B, encodes an inhibitory Gα protein that also affects appressorium development as mutants fail to produce appressorium and infection. However, the addition of AMP to the mutants restores the ability of the mutant. Mitogen-activated protein kinases also affect appressorium morphogenesis. There is a central signaling pathway that involves the protein PMK1. This influences appressorium development a great deal and mutants of it cannot produce appressoria or cause infection.

After it is formed, the appressorium develops enormous internal turgor pressure due to the glycerol it contains. During conidial germination, glycogen and lipids are degraded and, under the control of PMK1, they translocate rapidly to the germ tube tip. Lipolysis takes place rapidly during the generation of turgor pressure. In addition, spores contain trehalose, which seems to be required for turgor pressure and to be important for fungal infections. The mechanism by which turgor pressure is transformed into plant cuticle and cell wall penetration is not known yet. Gene *PLS1* encodes a tetraspanning protein, an unusual membrane-spanning protein, and seems to play a role in the regulation of penetration peg emergence.

Fusarium, *the Soilborne Plant Pathogen*

The genus *Fusarium* is a soilborne, necrotrophic, plant pathogenic fungus with many species that cause serious plant diseases around the world. *F. oxysporum* causes primarily vascular wilts on many crops, whereas numerous species, especially *F. solani*, cause root and stem rots and rots of seeds that are accompanied by the production of mycotoxins. A *Fusarium* species causing disease in immunocompromised human patients has been reported.

Fusarium oxysporum consists of more than 120 *formae specialis* according to the hosts they infect. Each of these can be subdivided into physiological races, each showing a characteristic pattern of virulence on differential host varieties. A gene-for-gene relationship appears to exist in many of the fungus race–host variety interactions. The fungus can survive in the soil as mycelium or as spores in the absence of its hosts. If a host is present, mycelium from germinating spores penetrates the host roots, enters the vascular system (xylem) in which it moves and multiplies, and causes the host to develop wilting symptoms. For the fungus to be successful in infecting the plant, it must mobilize different sets of genes for early plant–host signaling, attachment to root surface, enzymatic breakdown of physical barriers, defense against antifungal compounds of the host, and inactivation and death of host cells by fungal toxins.

Soil pH changes result in a transcription factor that activates alkaline-expressed genes and inhibits acid-expressed genes and thereby affect fungal cell growth, development, and possibly pathogenicity. Similarly, flavonoids and phytoalexins released by plant roots greatly affect the germination of fungal spores.

The early signals in plant–fungus recognition include transcription factor CTF1β. This mediates a constitutively expressed and starvation-activated cutinase gene (*cut2*) that release a few monomers of cutin from the plant. This triggers transcription of CTF1α. This mediates rapid activation of the fungal gene *cut1* and the latter secretes an extracellular cutinase that serves as a virulence factor.

Root attachment and penetration are under the control of a mitogen-activated protein kinase (MAPK). Once in contact with the root, the fungus needs to penetrate the cell walls. Several genes coding for cell wall-degrading pectinase and cellulase enzymes are activated sequentially. Pectin methyl esterases, pectin lyases, and polygalacturonases have been detected in vascular and other tissues and the respective genes have been identified. Several of the many genes coding for

hemicellulases and xylanases have also been found and isolated.

Once inside the plant, the fungus comes in contact with preexisting antimicrobial substances (phytoanticipins), such as the saponins α-tomatine in tomato and potato, α-chalconine and α-solanine in potato, and avenacin in oats. Different *formae specialis* of the fungus show inducible extracellular enzyme activities that cleave these substances into nontoxic molecules. Two other phytoanticipins, benzoxazolinone, produced by Gramineae, and acetophenone, produced by carnations, are also broken down by appropriate enzymes encoded by genes of the respective *Fusarium* special forms.

The fungus is also equipped to detoxify phytoalexins, as has been shown with the pea phytoalexin pisatin. Depending on their pisatin-demethylating ability, naturally occurring *Fusarium* isolates are either incapable of degrading pisatin (Pda2) or degrade it slowly (PdaL) or fast (PdaH), and their degrading ability paralleled their ability to cause disease. A gene, PDA1, responsible for the production of pisatin was identified some time ago, and five more pea pathogenicity (PEP) genes have been discovered as a cluster on the same chromosome as PDA1. Each of these genes alone increased virulence of the fungus on pea.

The fungus also adopts itself to the presence of lower levels of toxic materials. This is done by sterol-deficient mutants, which being resistant to saponins, react with sterols of the fungal cell membrane, or by developing a mechanism, that reduces pisatin retention in their cells.

Species of *Fusarium* not only inactivate toxic substances produced by the host, they also produce toxins of their own that increase their virulence. Some of the toxins, such as enniatin and fusaric acid, are phytotoxins, i.e., they are toxic to plants, whereas others, the mycotoxins, such as trichothecins and fumonisins, are toxic to animals. Disruption of production of enniatin by *F. avenacearum* and of fumonicin B1 by *F. moniliforme* greatly reduced the ability of the respective mutants to cause disease on potato tubers and maize seedlings, respectively.

Some species of *Fusarium*, e.g., *F. solani*, reproduce both sexually and asexually, whereas others, e.g., *F. oxysporum,* reproduce only asexually. Sexual reproduction, which leads to formation of a heterokaryon, is controlled by a set of *het* loci. The products of these loci lead to either vegetative compatibility or vegetative incompatibility, which leads to cell lysis after fusion of the hyphae. The mating type (MAT) is conferred by alternative alleles at the MAT locus. The latter consists of two functionally distinct alleles, MAT-1 and MAT-2. They encode proteins that bind to DNA, functioning as transcriptional regulators of genes required for sexual reproduction. The mating response is activated via a MAP kinase signal transduction pathway. In heterothallic *Fusarium* species, the MAT locus has three genes in MAT1-1 and one at MAT1-2. In homothallic species, all four genes are present close together on the same chromosome. In the asexual *F. oxysporum* species, field isolates contained either the MAT1-1 or the MAT1-2 genes, and the genes were highly similar to those of heterothallic species.

Ustilago maydis *and Corn Smut*

The genetics of the *U. maydis*–maize pathosystem has been studied extensively, especially as it pertains to fungal mating, morphogenesis, and fungal–plant interactions. The fungus begins its life cycle as a saprophytic haploid basidiospore that may produce short haploid mycelium and more cells by budding. Haploid cells can fuse and form a stable dikaryon if they carry different alleles of both the genetic loci a and b. Cell fusion is controlled by the mating-type locus, which has two alternative forms, a1 and a2. These control the cell/cell recognition and fusion events. After cell fusion, the subsequent steps in pathogenic development are controlled by the alleles in locus b. Production of a stable filamentous dikaryon and pathogenicity requires that the fungus be heterozygous at the multiallelic b locus. Mating compatible haploids produces a dikaryon, which is the pathogenic cell type. This is filamentous and an obligate biotroph. While heterozygosis at the b locus is required for pathogenicity, once mating has occurred the locus is no longer needed for pathogenicity, but its presence seems to slightly help the rate of gall formation. The dikaryotic filamentous hypha enters the plant cuticle and cell wall directly and causes a localized infection on maize plants that leads to the formation of large galls on any of the aboveground plant parts. Hyphae grow in the gall tissue intra- and intercellularly. When galls mature, nuclei of the dikaryon fuse and form the diploid teliospores. The teliospores disperse and germinate the following spring, producing promycelia (basidia), which undergo meiosis and produce budding haploid basidiospores.

Mating and pathogenicity are controlled by the master control genes a and b. At the locus, there are two distinct allelic sequences, a1 and a2. The a locus possesses two tightly linked genes, *mfa* and *pra* that encode, respectively, secreted pheromone and pheromone receptors that span the membrane. The pheromone encoded by *mfa* and the pheromone receptor of the *pra* genes of the opposite a mating type interact with each other, signaling the production by the *prfl gene* of a transcription factor that links the pheromone response pathway with the expression of the b locus and thus to pathogenicity. The *prfl* gene protein can activate at least two kinase

enzymes and is required for pathogenicity of the fungus due to its essential role in the regulation of the b mating type genes.

The b mating type locus encodes two proteins (bEast and bWest) that interact when produced by one of the 25 different alleles of each. The b locus controls events after cell fusion necessary for establishment of the infectious filamentous dikaryon and pathogenicity. Such interaction between bE1 and bW2 allele products establishes a novel regulatory protein that triggers formation of the infectious dikaryon. A switch controlled by a protein kinase dependent on cyclic AMP is important in the pathogenicity of *U. maydis*. Therefore a greater amount of PKA is required for initial plant infection and less for transition to gall formation and perhaps sporulation. Gall formation per se is not enough to trigger teliospore formation. One gene (hgl1) encodes a protein that is a transcription factor. Mutants of that gene produce large galls in maize kernels but the galls remain white because they do not form teliospores. Production of indole acetic acid (IAA) by the fungus has been suspected to be a factor in corn smut, and iad1, a gene encoding acetaldehyde hydrogenase, which converts indole-3-acetaldehyde to IAA, was isolated from *U. maydis*. Fungus mutants in this and another IAA gene produced a variety of IAA levels and a varying percentage of infective progeny.

BREEDING OF RESISTANT VARIETIES

The value of resistance in controlling plant disease was recognized in the early 1900s. Advances in the science of genetics and the obvious advantages of planting a resistant instead of a susceptible variety made the breeding of resistant varieties possible and desirable (Fig. 4-10). The more recent realization of the dangers of polluting the environment through chemical control of plant diseases gave additional impetus and importance to the breeding of resistant varieties. Thus, breeding resistant varieties, which is but one part of broader plant breeding programs, is more popular and more intensive today than it ever was in the past. Its usefulness and importance are paramount in the production of food and fiber. Nevertheless, some aspects of plant breeding, and of breeding resistant varieties in particular, have shown certain weaknesses and have allowed some plant disease epidemics to occur that could not have developed were it not for the uniformity created in crops through plant breeding.

Natural Variability in Plants

Today's cultivated crop plants are the result of selection, or selection and breeding, of plant lines that evolved naturally in one or many geographic areas of the world over millions of years. The evolution of plants from their ancient ancestors to present-day crop plants has occurred slowly and has produced countless genetically diverse forms of these plants. Many such plants still exist as wild types at the point of origin or in areas of natural spread of the plant. Although these plants may appear as useless remnants of evolution that are not likely to play a role in any future advances in agriculture, their diversity and survival in the face of the various pathogens that affect the crop in question indicate that they carry numerous genes for resistance against these pathogens.

Since the beginning of agriculture, some of the wild plants in each locality have been selected and cultivated and thus produced numerous cultivated lines or varieties. The most productive of these varieties were perpetuated in each locality from year to year, and those that survived the local climate and the pathogens continued to be cultivated. Nature and pathogens eliminated the weak and susceptible ones, while the farmers selected the best yielders among the survivors. Surviving varieties had different sets of major and minor genes for resistance. In this fashion, the selection of crop plants continued wherever they were grown, with people in each locality independently selecting varieties adapted to the local environment and resistant to local pathogens. Thus, numerous varieties of each crop plant were cultivated throughout the world and, by their own genetic diversity, contributed to make the crop locally adapted but, overall, genetically nonuniform and, thereby, safe from any sudden outbreak of a single pathogen over a large area.

Effects of Plant Breeding on Variability in Plants

During the 20th and 21st centuries, widespread, intensive, and systematic efforts have been made and continue to be made by plant breeders throughout the world to breed plants that combine the most useful genes for higher yields, better quality, uniform size of plants and fruit, uniform ripening, cold hardiness, and disease resistance. In searching for new useful genes, plant breeders cross existing, local, cultivated varieties with one another, with those of other localities, both here and abroad, and with wild species of crop plants from wherever they can be obtained. Furthermore, plant breeders often attempt to generate additional genetic variation by treating their plant material with mutagenic agents. More recently, plant breeders have been generating greater genetic variability and modifying or accelerating plant evolution in certain directions by various genetic engineering techniques. Using such techniques, plant

breeders can introduce genetic material (DNA) into plant cells directly via ballistic devices, via vectors (such as *A. tumefaciens*), or via protoplast fusion. Breeders can also obtain plants with different characteristics through culture and regeneration of somatic plant cells, by diploidization of haploid plants, and so on.

The initial steps in plant breeding generally increase the variability of genetic characteristics of plants in a certain locality by combining in such plants genes that were more or less widely separated by distance before. As breeding programs advance, however, and as several of the most useful genes are identified, subsequent steps in breeding tend to eliminate variability by combining the best genes in a few cultivated varieties and leaving behind or discarding plant lines that seem to have no usefulness at the time. In a short time a few "improved" varieties replace most or all others over large expanses of land. The most successful improved varieties are also adopted abroad and, before too long, some of them become popular worldwide and replace the numerous but commercially inferior local varieties. Occasionally, even the wild types themselves may be replaced by such a variety. Thus, Red Delicious apples, Elberta peaches, certain dwarf wheat and rice varieties, certain genetic lines of corn and potatoes, one or two types of bananas, and sugar cane are grown in huge acreages throughout the world. In almost every crop, relatively few varieties make up the great bulk of the cultivated acreage of the crop throughout a country or throughout the world. The genetic base of these varieties is often narrow, especially as many of them have been derived from crosses of the same or related ancestors. These few varieties are used so widely because they are the best available, they are stable and uniform, and therefore everybody wants to grow them. At the same time, however, because they are so widely cultivated, they carry with them not only the blessings but also the dangers of uniformity. The most serious of these dangers is the vulnerability of large uniform plantings to sudden outbreaks of catastrophic plant disease epidemics.

Plant Breeding for Disease Resistance

Most plant breeding is done for the development of varieties that produce greater yields or better quality. While such varieties are being developed, they are tested for resistance against some of the most important pathogens present in the area where the variety is developed and where it is expected to be cultivated. If the variety is resistant to these pathogens, it may be released to growers for immediate production. If, however, it is susceptible to one or more of the pathogens, the variety is usually shelved or discarded (Fig. 4-17); sometimes it is released for production if the pathogen can be controlled by other means, such as with chemicals, but more often it is subjected to further breeding in an attempt to incorporate into the variety genes that would make it resistant to the pathogens without changing any of its desirable characteristics.

Sources of Genes for Resistance

The source of genes for resistance is the same gene pool of the crop that provides genes for every other inherited characteristic, namely, other native or foreign commercial varieties, older varieties abandoned earlier or discarded breeders' stock, wild plant relatives, and, occasionally, induced mutations. Often, genes of resistance are present in the varieties or species normally grown in the area where the disease is severe and in which the need for resistant varieties is most pressing. With most diseases, a few plants remain virtually unaffected by the pathogen, although most or all other plants in the area may be severely diseased. Such survivor plants are likely to have remained healthy because of resistant characteristics present in them (Fig. 4-18).

If no resistant plants can be found within the local population of the species, plants of the same species from other areas and plants of other species (cultivated or wild) are checked for resistance. If resistant plants are found, they are crossed with the cultivated varieties in an effort to incorporate the resistance genes of the other species into the cultivated varieties. With some diseases, such as late blight of potatoes, it has been necessary to look for resistance genes in species growing in the area where the disease originated. Presumably, plants existing in those areas managed to survive the long, continuous presence of the pathogen because of their resistance to it.

Techniques Used in Classical Breeding for Disease Resistance

The same methods used to breed for any heritable characteristic are also used for breeding for disease resistance and depend on the mating system of the plant (self- or cross-pollinated). Breeding for disease resistance, however, is considerably more complicated. The reason is that resistance can be assayed only by making the plants diseased, i.e., by employing another living and variable organism that must interact with the plants. In recent years, however, molecular markers associated with resistance-related enzymes, phenolics, and other compounds have been used in effectively selecting for resistance in place of inoculating the plants with the pathogen, at least in the early stages of breeding. Breeding for resistance is also complicated because resistance

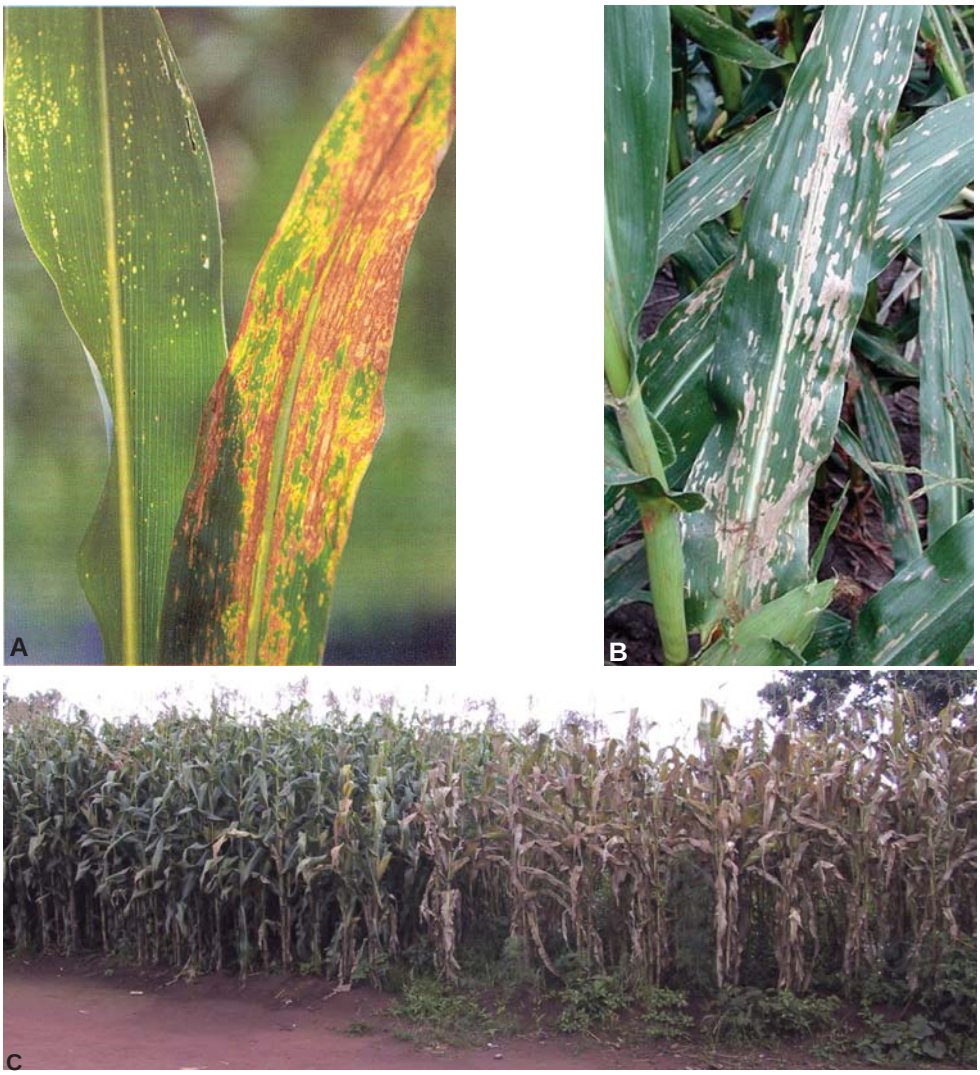

FIGURE 4-17 Examples of resistant and susceptible corn plants. (A) Leaves of resistant (left) and susceptible (right) plants infected with the corn leaf blight fungus. (B) Lesions of gray leaf spot on a corn plant. (C) A resistant corn hybrid (left) and a resistant one to gray leaf spot caused by the fungus *Cercospora zeae-maydis*. (Photo A courtesy USDA, B & C courtesy R. Asiedu, International Instit. Tropical Agriculture).

may not be stable and may break down under certain conditions. For these reasons, several more or less sophisticated systems of screening for resistance have been developed. These screening systems include (1) precise conditions for inoculating the plants with the pathogen, (2) accurate monitoring and control of the environmental conditions in which the inoculated plants are kept, and (3) accurate assessment of disease incidence (percentage of plants, leaves, or fruits infected) and disease severity (proportion of the total area of plant tissue affected by disease). The following techniques are the main ones used for breeding disease resistance.

Seed, Pedigree, and Recurrent Selection

Mass selection of seed from the most highly resistant plants surviving in a field where natural infection occurs regularly is a simple method but improves plants only slowly. Moreover, in cross-pollinated plants there is no control of pollen source.

In pure line or pedigree selection, individual highly resistant plants and their progenies are propagated separately and are inoculated repeatedly to test for resistance. This method is easy and most effective with self-pollinated crops, but it is quite difficult with cross-pollinated ones.

FIGURE 4-18 While most of these staked yam plants were killed or nearly killed by the yam anthracnose fungus *Colletotrichum gloeosporioides*, several plants survive, despite the overwhelming amount of fungus inoculum around them due to genes for resistance they carry.

In recurrent selection or backcrossing, a desirable but susceptible variety of a crop is crossed with another cultivated or wild relative that carries resistance to a particular pathogen. The progeny is then tested for resistance, and the resistant individuals are backcrossed to the desirable variety. This is repeated several times until the resistance is stabilized in the genetic background of the desirable variety. This method is time-consuming and its effectiveness varies considerably with each particular case. It can be applied somewhat more easily in cross-pollinated than in self-pollinated crops.

Other Techniques

Other classical breeding techniques for disease resistance include the use of F_1 hybrids of two different but homozygous lines carrying different genes for resistance, which allows one to take advantage of the phenomenon of heterosis (hybrid vigor); use of natural or artificially induced (UV light, X rays) mutants that show increased resistance; and change of the number of chromosomes in a plant and production of euploids (4N, 6N) or aneuploids ($2N \pm 1$ or 2 chromosomes) using chemicals such as colchicine and by radiation.

Breeding for Resistance Using Tissue Culture and Genetic Engineering Techniques

Advances in plant tissue culture include meristem tip propagation, callus and single cell culture, haploid plant production, and protoplast isolation, culture, transformation, fusion, and regeneration into whole plants. These advances have opened up a whole new array of possibilities and methodologies for plant improvement, including improvement of plant resistance to infection by pathogens. The potential of these techniques is further augmented by combination with molecular technologies (genetic engineering). Genetic engineering techniques allow the detection, isolation, modification, transfer, and expression of single genes, or groups of related genes, from one organism to another. Several tissue culture techniques, e.g., regeneration of whole plants from calluses, single cells, protoplasts, and microspores or pollen, lead by themselves to plants showing greater variability in many characteristics, including resistance to disease. Selection of the best among such plants and subsequent application of classical breeding techniques make possible the production of improved plants with greater efficiency and at a much greater rate. The application of genetic engineering technologies in plant improvement depends on the kinds of plant tissue culture with which one is working, but it increases their potential tremendously by enabling plant scientists to pinpoint cell genes with specific functions and to transfer them into new cells and organisms.

Tissue Culture of Disease-Resistant Plants

Tissue culture of disease-resistant plants is particularly useful with clonally propagated plants such as strawberries, apples, bananas, sugar cane, cassava, and potatoes. Prolific plantlet production from meristem and other tissue cultures facilitates the rapid propagation of plants with exceptional (resistant) genotypes, especially in those crops not propagated easily by seed. An even greater use of tissue culture is for the production of pathogen-free stocks of clonally propagated susceptible plants.

Isolation of Disease-Resistant Mutants from Plant Cell Cultures

Plants regenerated from culture (calluses, single cells, or protoplasts) often show considerable variability (**somaclonal variation**), much of it useless or deleterious. However, plants with useful characteristics may also emerge. For example, when plants were regenerated from leaf protoplasts of a potato variety susceptible to both *Phytophthora infestans* and *Alternaria solani*, some of them (5 of 500) were resistant to *A. solani* and some (20 of 800) were resistant to *P. infestans*. Similarly, plants exhibiting increased resistance to disease caused by *Cochliobolus* and *Ustilago* were obtained from tissue cultures of sugar cane.

Production of Resistant Dihaploids from Haploid Plants

Immature pollen cells (microspores), and less often megaspores, of many plants can be induced to develop into haploid (1N) plants in which single copies (alleles) of each gene are present in all sorts of combinations. By vegetative propagation and proper screening for disease resistance, the most highly resistant haploids can be selected. These haploids can be subsequently treated with colchicine, which results in diploidization of the nuclei, i.e., doubling of the number of chromosomes and the production of dihaploid plants homozygous for all genes, including genes for resistance.

Increasing Disease Resistance by Protoplast Fusion

Protoplasts from closely related and even from unrelated plants, under proper conditions, can be made to fuse. The fusion produces **hybrid cells** containing the nuclei (chromosomes) and the cytoplasm of both protoplasts or it might result in **cybrid cells** containing the nucleus of one cell and the cytoplasm of the other cell. Generally, hybrids of unrelated cells sooner or later abort or may produce calluses, but they do not regenerate plants. In combinations of more or less related cells, however, although many or most of the chromosomes of one of the cells are eliminated during cell division, one or a few chromosomes of that cell survive and may be incorporated in the genome of the other cell. In this way, plants with more chromosomes and thereby new characteristics can be regenerated from the products of protoplast fusion. Protoplast fusion is particularly useful between protoplasts of different, highly resistant haploid lines of the same variety or species. Protoplast fusion of such lines results in diploid plants that combine the resistance genes of two highly resistant haploid lines.

Genetic Transformation of Plant Cells for Disease Resistance

Genetic material (DNA) can be introduced into plant cells or protoplasts by several methods. Such methods include direct DNA uptake, microinjection of DNA, liposome (lipid vesicle)-mediated delivery of DNA, delivery by means of centromere plasmids (minichromosomes), use of plant viral vectors, and, most importantly, bombardment of cells with tiny spheres carrying DNA and by use of the natural gene vector system of *A. tumefaciens*, the cause of crown gall disease of many plants. In all of these methods, small or large pieces of DNA are introduced into plant cells or protoplasts, and the DNA may be integrated in the plant chromosomal DNA. When the introduced DNA carries appropriate regulatory genes recognized by the plant cell or is integrated near appropriate regulatory genes along plant chromosomes, the DNA is "expressed," i.e., it is transcribed into mRNA, which is then translated into protein.

So far, only microprojectile bombardment and the *Agrobacterium* system have been used successfully to introduce into plants specific new genes that were then expressed by the plant. This was accomplished by isolating several genes of interest from plants or pathogens and splicing them into appropriate plasmids. These were subsequently used to coat the surface of tiny spheres, which were bombarded into plant cells or were introduced into a disarmed Ti plasmid of *Agrobacterium*; bacteria were then allowed to infect appropriate other plants. On infection, about one-tenth of the DNA of the plasmid, containing the new gene, is transferred to the plant cell and is incorporated into the plant genome. There, the new gene replicates during plant cell division and is expressed along with the other plant genes.

To date, several dozen R genes for disease resistance have been isolated, and several kinds of plants have been transformed genetically for disease resistance. This has been accomplished for fungal, bacterial, and viral host–pathogen combinations. In addition, viral, bacterial, fungal, or plant genes, when introduced into plants via genetic engineering techniques, provided various degrees of resistance (pathogen-derived resistance) in the plant to the pathogen from which the gene or DNA fragment was obtained and also to other pathogens. It is generally expected that breeding for disease resistance will quickly profit greatly from the application of techniques in genetic engineering.

Genetic engineering of plants for disease resistance is now used in practice with several crops. The best-documented cases involve plants engineered for resistance to viruses, such as cucurbits engineered for resistance to *cucumber mosaic*, *watermelon mosaic*, and *zucchini yellow mosaic viruses*; papaya engineered for resistance to *papaya ringspot virus*; potato engineered for resistance to *potato leaf roll* and *potato Y viruses*; and wheat engineered for resistance to *wheat streak mosaic virus*. More examples and details of genetic engineering of plants for disease resistance can be found in Chapter 6.

Advantages and Problems in Breeding for Vertical or Horizontal Resistance

Resistance may be obtained by incorporating one, a few, or many resistance genes into a variety. Some of these

genes may control important steps in disease development and may therefore play a major role in disease resistance. Other genes may control peripheral events of lesser importance in disease development and, therefore, play a relatively minor role in disease resistance. Obviously, one or a few major role genes could be sufficient to make a plant resistant to a pathogen (R-gene, monogenic, oligogenic, or vertical resistance). However, it would take many minor effect genes to make a plant resistant (polygenic or horizontal resistance). More importantly, whereas a plant with vertical resistance may be completely resistant to a pathogen, a plant with horizontal resistance is never completely resistant or completely susceptible. Furthermore, vertical resistance is easy to manipulate in a breeding program, including the application of genetic engineering techniques, and therefore is often preferred to horizontal resistance. However, both vertical and horizontal resistances have their advantages and limitations.

Vertical resistance is aimed against specific pathogens or pathogen races. Vertical resistance is most effective when (1) it is incorporated in annual crops that are easy to breed, such as small grains; (2) it is directed against pathogens that do not reproduce and spread rapidly, such as *Fusarium*, or pathogens that do not mutate very frequently, such as *Puccinia graminis*; (3) it consists of "strong" genes (R-genes) that confer complete and long-term protection to the plant that carries it; and (4) the host population does not consist of a single genetically uniform variety grown over large acreages. If one or more of these, and several other, conditions are not met, vertical resistance becomes short lived, i.e., it breaks down as a result of the appearance of new pathogen races that can bypass or overcome it.

Horizontal resistance confers incomplete (partial) but more durable protection: it does not break down as quickly and suddenly as most vertical resistance. Horizontal resistance involves many host physiological processes that act as mechanisms of defense and that are beyond the limits of the capacity of the pathogen to change, i.e., beyond the probable limits of its variability. Horizontal resistance is present universally in wild and domesticated plants and operates against all races of a pathogen, including the most pathogenic ones. Varieties with horizontal (polygenic, general, or nonspecific) partial resistance remain resistant much longer than varieties with vertical (oligogenic or specific) resistance, but the level of resistance in plants with horizontal resistance is much lower than in plants with vertical resistance.

Because varieties with vertical resistance are often attacked suddenly and rapidly by a new virulent race and lead to severe epidemics, various strategies have been developed to avoid these disadvantages. In some crops this has been accomplished through the use of multilines or by pyramiding. **Multilines** are mixtures of individual varieties (lines or cultivars) that are agronomically similar but differ in their resistance genes. **Pyramiding** consists of using varieties that are derived from crossing several to many varieties that contain different resistance genes and then selecting from them those that contain the mixtures of genes. Multilines and pyramiding have been developed mostly in small grains against the rust fungi, but their use is likely to increase in these and other crops as the control of plant diseases with specific resistance and with chemicals becomes more risky or less acceptable.

Incorporating genes for resistance from wild or unsatisfactory plants into susceptible but agronomically desirable varieties is a difficult and painstaking process involving repeated crossings, testings, and backcrossings to the desirable varieties. The feasibility of the method in most cases, however, has been proved repeatedly. Through breeding, varieties of some crops have been developed in which genes for resistance against several different diseases have been incorporated.

Vulnerability of Genetically Uniform Crops to Plant Disease Epidemics

Varieties with even complete vertical resistance do not remain resistant forever. The continuous production of mutants and hybrids in pathogens sooner or later leads to the appearance of races that can infect previously resistant varieties. Sometimes, races may exist in an area in small populations and avoid detection until after the introduction of a new variety or virulent races of the pathogen existing elsewhere may be brought in after introduction of the resistant variety. In all cases, widespread cultivation of a single, previously resistant variety provides an excellent substrate for the rapid development and spread of the new race of the pathogen, and it usually leads to an epidemic. Thus, genetic uniformity in crops, although very desirable when it concerns horticultural characteristics, is undesirable and often catastrophic when it occurs in the genes of resistance to diseases.

The cultivation of varieties with genetically uniform disease resistance is possible and quite safe if other means of plant disease control, such as chemical, are possible. Thus, a few fruit tree varieties, such as Red Delicious apples, Bartlett pears, Elberta peaches, and navel oranges, are cultivated throughout the world in the face of numerous virulent fungal and bacterial pathogens that would destroy them in a short time were it not for the fact that the trees are protected from the pathogens by numerous chemical sprays annually. Even such varieties, however, suffer tremendous losses when

affected by pathogens that cannot be controlled with chemicals, as in the case of fire blight of pears, pear decline, and tristeza disease of citrus.

Another case in which varieties with genetically uniform disease resistance are not likely to suffer from severe disease epidemics is when the resistance is aimed against slow-moving soil pathogens such as *Fusarium* and *Verticillium*. Aside from the fact that some pathogens normally produce fewer races than others, even if new races are produced at the same rate, soilborne pathogens lack the dispersal potential of airborne ones. As a result, a new race of a soilborne pathogen would be limited to a relatively small area for a long time, and although it could cause a locally severe disease, it would not spread rapidly and widely to cause an epidemic. The slow spread of such virulent new races of soilborne pathogens allows time for the control of the disease by other means or the replacement of the variety with another one resistant to the new race.

Genetic uniformity in plant varieties becomes a serious disadvantage in the production of major crops because of the potential danger of sudden and widespread disease epidemics caused by airborne or insectborne pathogens in the vast acreages in which each of these varieties is often grown. Several examples of epidemics that resulted from genetic uniformity are known and some of them have already been mentioned. Southern corn leaf blight was the result of the widespread use of corn hybrids containing the Texas male-sterile cytoplasm; the destruction of the 'Ceres' spring wheat by race 56 of *Puccinia graminis*; and of 'Hope' and its relative bread wheats by race 15B of *P. graminis*, were all the result of replacement of numerous genetically diverse varieties by a few uniform ones. *The Cochliobolus* (*Helminthosporium*) blight of Victoria oats was the result of replacing many varieties with the rust-resistant Victoria oats; coffee rust destroyed all coffee trees in Ceylon because all of them originated from uniform susceptible stock of *Coffea arabica*; and tristeza continues to destroy millions of orange trees in South, Central, and North America because they were propagated on hypersensitive resistant sour orange rootstocks.

Despite these and many other well-known examples of plant disease epidemics that occurred because of the concentrated cultivation of genetically uniform crops over large areas, crop production continues to depend on genetic uniformity. A few varieties of each crop used to, and for some crops still, make up the bulk of the cultivated crop over as vast an area as the United States. Although a relatively large number of varieties are available for each crop, only a few varieties, often two or three, are grown in more than half the acreage of each crop, and in some they make up more than three-fourths of the crop. For example, two pea varieties make up almost the entire pea crop of the country (96%), i.e., about 400,000 acres, and two varieties account for 42% of the sugar beet crop, i.e., about 600,000 acres. The figures become even more spectacular when one considers the most popular varieties of the truly large acreage crops. Thus, although six corn varieties (hybrids) account for 71%, or 47 million acres, one of them alone accounts for 26%, or 17 million acres. Similarly, six varieties of soybean account for about 24 million acres of that crop, and most of these varieties share common ancestors.

It is apparent that several hundreds of thousands or several million acres planted to one variety present a huge opportunity for the development of an epidemic. The variety, of course, is planted so widely because it is resistant to existing pathogens. However, this resistance puts extreme survival pressure on the pathogens over that area. It takes one "right" change in one of the zillions of pathogen individuals in the area to produce a new virulent race that can attack the variety. When that happens it is a matter of time — and, usually, of favorable weather — before the race breaks loose, the epidemic develops, and the yield of the variety is destroyed or reduced below acceptable economic levels. In some cases the appearance of the new race is detected early and the variety is replaced with another one, resistant to the new race, before a widespread epidemic occurs; this, of course, requires that varieties of a crop with a different genetic base are available at all times. For this reason, most varieties must usually be replaced within about 3 to 5 years from the time of their widespread distribution.

In addition to the genetic uniformity within one variety, plant breeding often introduces genetic uniformity to several or all cultivated varieties of a crop by introducing one or several genes in all of these varieties or by replacing the cytoplasm of the varieties with a single type of cytoplasm. Induced uniformity through introduced genes includes, for example, the seedless condition in grapes and watermelons, the dwarfism gene in the dwarf wheat and rice varieties, the monogerm gene in sugar beet varieties, the determinate gene in tomato varieties, and the stringless gene in bean varieties. Uniformity through replacement of the cytoplasm occurred, of course, in most corn hybrids in the later 1960s when the Texas male-sterile cytoplasm replaced the normal cytoplasm. Cytoplasmic uniformity is also employed commercially in several varieties of sorghum, sugar beet, and onions; it is studied in wheat and is also present in cotton and cantaloupe. Neither the introduced genes nor the replacement cytoplasm, of course, makes the plant less resistant to diseases, but if a pathogen appears that is favored by or can take advantage of the characters controlled by that gene or other genes linked to it or by

genes in that cytoplasm, then the stage is set for a major epidemic. That this can happen was proved by the southern corn leaf blight epidemic of 1970 and by the susceptibility of dwarf wheats to new races of *Septoria* and *Puccinia*, of tomatoes with the determinate gene to *Altenaria*, and others.

In more recent years, efforts have been made to plant a smaller percentage of the total acreage of a crop with a few selected varieties, but for most crops and most areas that acreage is still too great. For example, in the mid-1990s the top six soybean varieties and the top nine wheat varieties made up only 41 and 34% of the total soybean and wheat acreage, respectively. However, the three most popular cotton varieties made up 54% of the total cotton acreage. Furthermore, the four most popular potato varieties in each of the 11 leading potato-producing states accounted for 63 to 100% of the total potato crop in any one of these states, and the most popular barley varieties in the top six barley-producing states accounted for 44 to 94% of the total crop in each state.

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chapter five

HOW PATHOGENS ATTACK PLANTS

The intact, healthy plant is a community of cells built in a fortress-like fashion. Plant cells consist of cell wall, cell membranes, and cytoplasm, which contains the nucleus and various organelles (Fig. 5-1) and all the substances for which the pathogens attack them. The cytoplasm and the organelles it contains are separated from each other by membranes that carry various types of proteins embedded in them (Fig. 5-2). The plant surfaces that come in contact with the environment either consist of cellulose, as in the epidermal cells of roots and in the intercellular spaces of leaf parenchyma cells, or consist of a cuticle that covers the epidermal cell walls, as is the case in the aerial parts of plants. Often an additional layer, consisting of waxes, is deposited outside the cuticle, especially on younger parts of plants (Fig. 5-3).

Pathogens attack plants because during their evolutionary development they have acquired the ability to live off the substances manufactured by the host plants, and some of the pathogens depend on these substances for survival. Many substances are contained in the protoplast of the plant cells, however, and if pathogens are to gain access to them they must first penetrate the outer barriers formed by the cuticle and/or cell walls. Even after the outer cell wall has been penetrated, further invasion of the plant by the pathogen necessitates the penetration of more cell walls. Furthermore, the plant cell contents are not always found in forms immediately utilizable by the pathogen and must be broken down to units that the pathogen can absorb and assimilate. Moreover, the plant, reacting to the presence and activities of the pathogen, produces structures and chemical substances that interfere with the advance or the existence of the pathogen; if the pathogen is to survive and to continue living off the plant, it must be able to overcome such obstacles.

Therefore, for a pathogen to infect a plant it must be able to make its way into and through the plant, obtain nutrients from the plant, and neutralize the defense reactions of the plant. Pathogens accomplish these activities mostly through secretions of chemical substances that affect certain components or metabolic mechanisms of

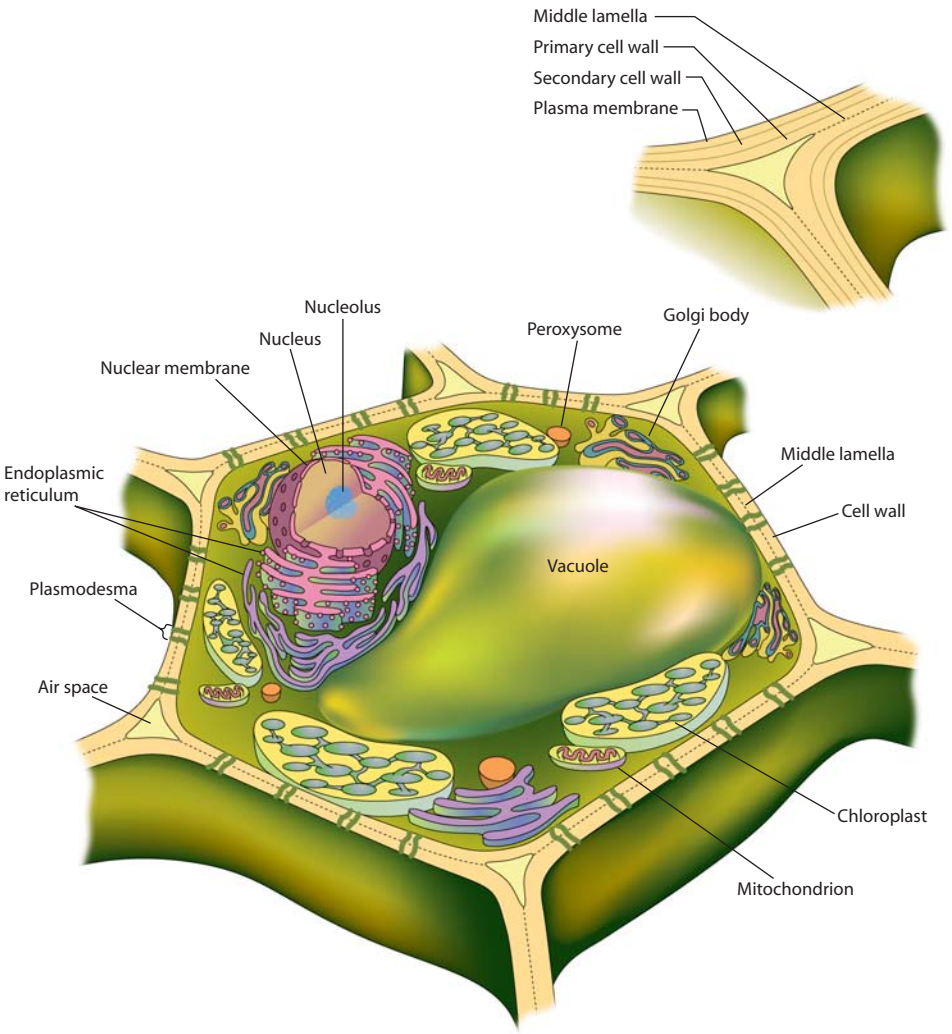


FIGURE 5-1 Schematic representation of a plant cell and its main components.

their hosts. Penetration and invasion, however, seem to be aided by, or in some cases be entirely the result of, the mechanical force exerted by certain pathogens on the cell walls of the plant.

MECHANICAL FORCES EXERTED BY PATHOGENS ON HOST TISSUES

Plant pathogens are, generally, tiny microorganisms that cannot apply a "voluntary" force to a plant surface. Only some fungi, parasitic higher plants, and nematodes appear to apply mechanical pressure to the plant surface they are about to penetrate. The amount of pressure, however, may vary greatly with the degree of "presoftening" of a plant surface by enzymatic secretions of the pathogen.

For fungi and parasitic higher plants to penetrate a plant surface, they must, generally, first adhere to it. Hyphae and radicles are usually surrounded by mucilaginous substances, and their adhesion to the plant seems to be brought about primarily by the intermolecular forces developing between the surfaces of plant and pathogen on close contact with the adhesive substances and with one another. In some cases an adhesion pad forms from the spore when it comes in contact with a moist surface, and cutinase and cellulase enzymes released from the spore surface help the spore adhere to the plant surface. Spores of some fungi carry adhesive substances at their tips that, on hydration, allow spores to become attached to various surfaces.

After contact is established, the diameter of the tip of the hypha or radicle in contact with the host increases and forms the flattened, bulb-like structure called the appressorium (Figs. 2-4 and 2-5). This increases the area of adherence between the two organisms and securely fastens the pathogen to the plant. From the appressorium, a fine growing point, called the penetration peg,

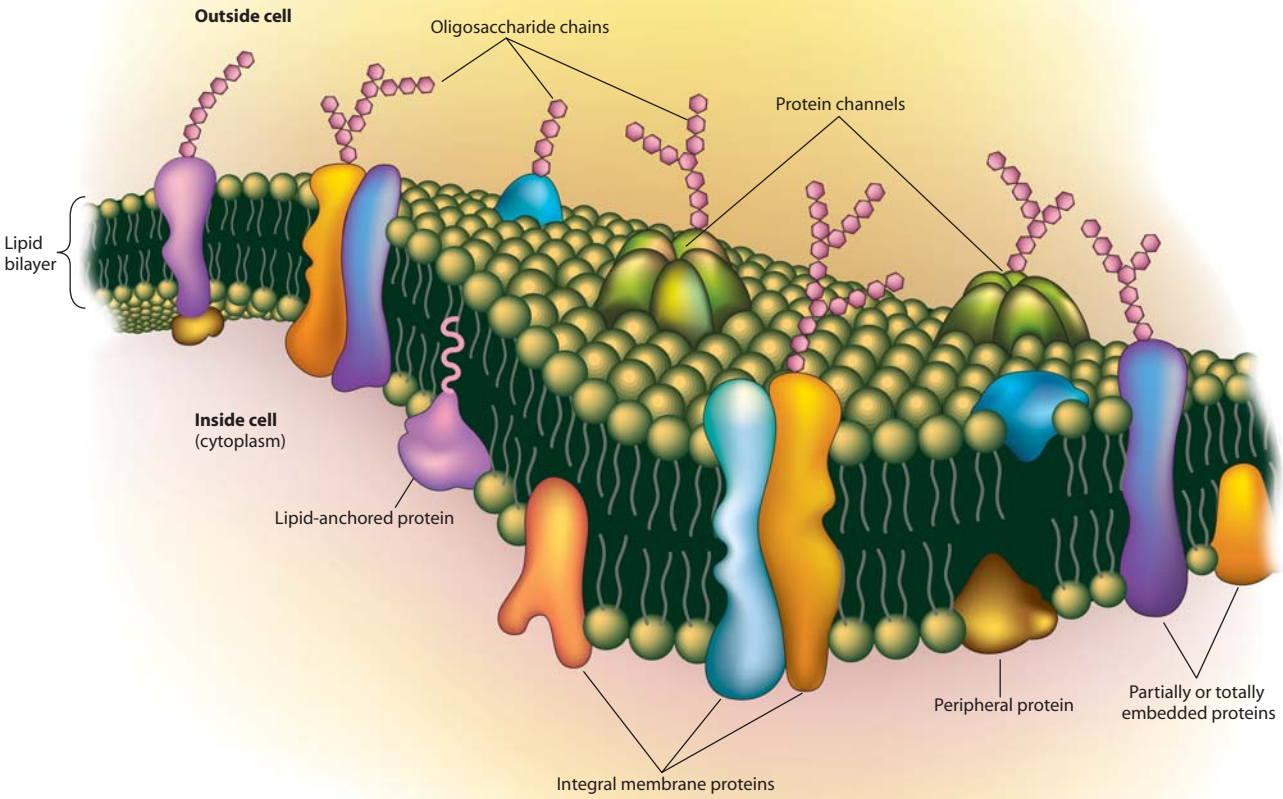


FIGURE 5-2 Schematic representation of a portion of a cell membrane and of the arrangement of protein molecules in relation to the membrane.

arises and advances into and through the cuticle and cell wall. In some fungi, such as *Alternaria*, *Cochliobolus*, *Colletotrichum*, *Gaeumannomyces*, *Magnaporthe*, and *Verticillium*, penetration of the plant takes place only if melanin (dark pigment) accumulates in the appressorial cell wall. It appears that melanin produces a rigid structural layer and, by trapping solutes inside the appressorium, causes water to be absorbed. This increases the turgor pressure in the appressorium and, thereby, the physical penetration of the plant by the penetration peg. If the underlying host wall is soft, penetration occurs easily. When the underlying wall is hard, however, the force of the growing point may be greater than the adhesion force of the two surfaces and may cause separation of the appressorial and host walls, thus averting infection. Penetration of plant barriers by fungi and parasitic higher plants is almost always assisted by the presence of enzymes secreted by the pathogen at the penetration site, resulting in the softening or dissolution of the barrier. It was found, for example, that while appressoria of some powdery mildew fungi developed a maximum turgor pressure of 2–4 MPa, approximately sufficient to bring about host cell penetration, two cellulases were also present: one primarily at the tip of the appressorial germ tube and the other at the tip of the primary germ tube.

While the penetration tube is passing through the cuticle, it usually attains its smallest diameter and

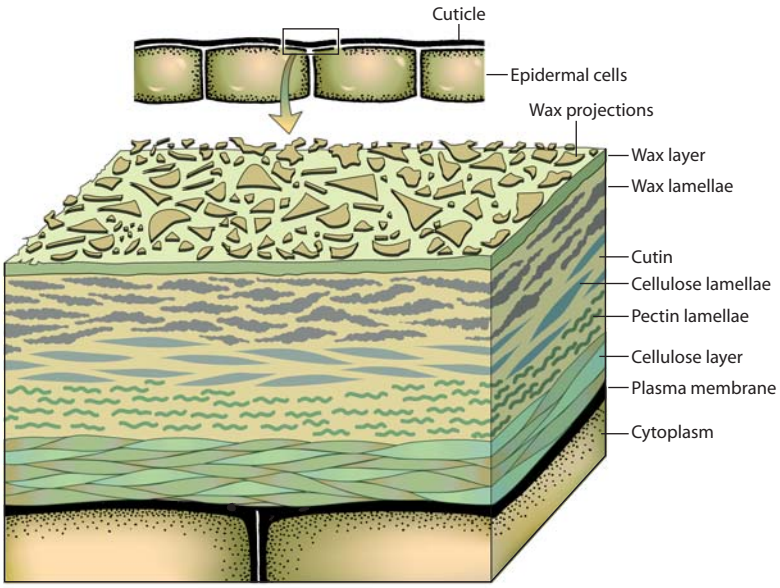


FIGURE 5-3 Schematic representation of the structure and composition of the cuticle and cell wall of foliar epidermal cells. [Adapted from Goodman *et al.* (1967).]

appears thread-like. After penetration of the cuticle, the hyphal tube diameter often increases considerably. The penetration tube attains the diameter normal for the hyphae of the particular fungus only after it has passed through the cell wall (see Figs. 2-5 and 2-9 in Chapter 2).

Nematodes penetrate plant surfaces by means of the stylet, which is thrust back and forth and exerts mechanical pressure on the cell wall (Fig. 2-10). The nematode first adheres to the plant surface by suction, which it develops by bringing its fused lips in contact with the plant. After adhesion is accomplished, the nematode brings its body, or at least the forward portion of its body, to a position vertical to the cell wall. With its head stationary and fixed to the cell wall, the nematode then thrusts its stylet forward while the rear part of its body sways or rotates slowly round and round. After several consecutive thrusts of the stylet, the cell wall is pierced, and the stylet or the entire nematode enters the cell.

Once a fungus or nematode has entered a cell, it generally secretes increased amounts of enzymes that presumably soften or dissolve the opposite cell wall and make its penetration easier. Mechanical force, however, probably is brought to bear in most such penetrations, although to a lesser extent.

Considerable mechanical force is also exerted on host tissues from the inside out by some pathogenic fungi on formation of their fructifications in the tissues beneath the plant surface. Through increased pressure, the sporophore hyphae, as well as fruiting bodies, such as pycnidia and perithecia, push outward and cause the cell walls and the cuticle to expand, become raised in the form of blister-like proturberances, and finally break.

CHEMICAL WEAPONS OF PATHOGENS

Although some pathogens may use mechanical force to penetrate plant tissues, the activities of pathogens in plants are largely chemical in nature. Therefore, the effects caused by pathogens on plants are almost entirely the result of biochemical reactions taking place between substances secreted by the pathogen and those present in, or produced by, the plant.

The main groups of substances secreted by pathogens in plants that seem to be involved in production of disease, either directly or indirectly, are enzymes, toxins, growth regulators, and polysaccharides (plugging substances). These substances vary greatly as to their importance in pathogenicity, and their relative importance may be different from one disease to another. Thus, in some diseases, such as soft rots, enzymes seem to be by far the most important, whereas in diseases such as crown gall, growth regulators are apparently the main substances involved. However, in the *Bipolaris* blight of Victoria oats, the disease is primarily the result of a toxin secreted in the plant by the pathogen. Enzymes, toxins, and growth regulators, probably in that order, are considerably more common and probably more important in plant disease development than polysaccharides. It has also been shown that some pathogens

produce compounds that act as suppressors of the defense responses of the host plant.

Among the plant pathogens, all except viruses and viroids can probably produce enzymes, growth regulators, and polysaccharides. How many of them produce toxins is unknown, but the number of known toxin-producing plant pathogenic fungi and bacteria increases each year. Plant viruses and viroids are not known to produce any substances themselves, but they induce the host cell to produce either excessive amounts of certain substances already found in healthy host cells or substances completely new to the host. Some of these substances are enzymes, and others may belong to one of the other groups mentioned earlier.

Pathogens produce these substances either in the normal course of their activities (constitutively) or when they grow on certain substrates such as their host plants (inducible). Undoubtedly, natural selection has favored the survival of pathogens that are assisted in their parasitism through the production of such substances. The presence or the amount of any such substance produced, however, is not always a measure of the ability of the pathogen to cause disease. It must also be kept in mind that many substances, identical to those produced by pathogens, are also produced by the healthy host plant.

In general, plant pathogenic enzymes disintegrate the structural components of host cells, break down inert food substances in the cell, or affect components of its membranes and the protoplast directly, thereby interfering with its functioning systems. Toxins seem to act directly on protoplast components and interfere with the permeability of its membranes and with its function. Growth regulators exert a hormonal effect on the cells and either increase or decrease their ability to divide and enlarge. Polysaccharides seem to play a role only in the vascular diseases, in which they interfere passively with the translocation of water in the plants.

Enzymes in Plant Disease

Enzymes are generally large protein molecules that catalyze organic reactions in living cells and in solutions. Because most kinds of chemical reaction that occur in a cell are enzymatic, there are almost as many kinds of enzymes as there are chemical reactions. Each enzyme, being a protein, is coded for by a specific gene. Some enzymes are present in cells at all times (constitutive). Many are produced only when they are needed by the cell in response to internal or external gene activators (induced). Each type of enzyme often exists in several forms known as isozymes that carry out the same function but may vary from one another in several properties, requirements, and mechanism of action.

Enzymatic Degradation of Cell Wall Substances

Usually, the first contact of pathogens with their host plants occurs at a plant surface. Aerial plant part surfaces consist primarily of cuticle and/or cellulose, whereas root cell wall surfaces consist only of cellulose. Cuticle consists primarily of cutin, more or less impregnated with wax and frequently covered with a layer of wax. The lower part of cutin is intermingled with pectin and cellulose lamellae and lower yet there is a layer consisting predominantly of pectic substances; below that there is a layer of cellulose. Polysaccharides of various types are often found in cell walls. Proteins of many different types, both structural, e.g., elastin, which helps loosen the cell wall, and extensin, which helps add rigidity to the cell wall, some enzymes, and some signal molecules that help receive or transmit signals inward or outward, are normal constituents of cell walls. Finally, epidermal cell walls may also contain suberin and lignin. The penetration of pathogens into parenchymatous tissues is facilitated by the breakdown of the internal cell walls, which consist of cellulose, pectins, hemicelluloses, and structural proteins, and of the middle lamella, which consists primarily of pectins. In addition, complete plant tissue disintegration involves the breakdown of lignin. The degradation of each of these substances is brought about by the action of one or more sets of enzymes secreted by the pathogen.

Cuticular Wax

Plant waxes are found as granular, blade, or rod-like projections or as continuous layers outside or within the cuticle of many aerial plant parts (Fig. 5-4). The presence and condition of waxes at the leaf surface affect the degree of colonization of leaves and the effect varies with the plant species. Electron microscope studies suggest that several pathogens, e.g., *Puccinia hordei*, produce enzymes that can degrade waxes. Another fungus, *Pestalotia malicola*, which attacks fruit of Chinese quince, grows on, within, and beneath the fruit cuticle. Fungi and parasitic higher plants, however, apparently can penetrate wax layers by means of mechanical force alone.

Cutin

Cutin is the main component of the cuticle. The upper part of the cuticle is admixed with waxes, whereas its lower part, in the region where it merges into the outer walls of epidermal cells, is admixed with pectin and cellulose (see Fig. 5-3). Cutin is an insoluble polyester of C_{16} and C_{18} hydroxy fatty acids.

Many fungi and a few bacteria have been shown to produce cutinases and/or nonspecific esterases, i.e.,

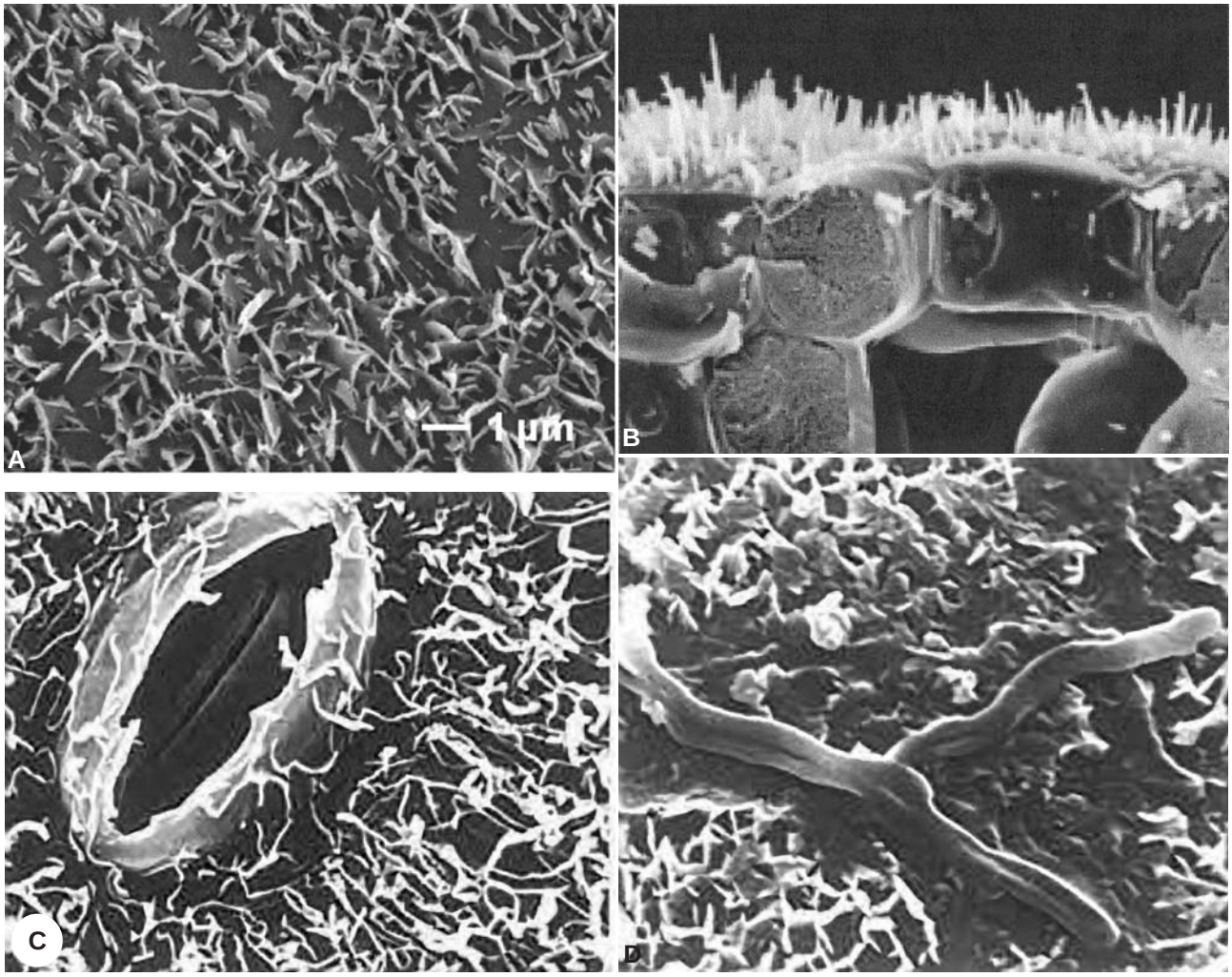


FIGURE 5-4 Morphology of cuticular wax projections on different leaf surfaces. (A) Surface view of wax on corn leaf. (B) Wax projections as seen in cross section of leaf. (C) Wax projections surrounding a stoma. (D) Wax degraded along the passage of fungal mycelium. [Photographs courtesy of (A) L. M. Marcell and G. A. Beattie, Iowa State University, (B) H. V. Davis, United Kingdom, (C and D) P. V. Sangbusen, Hamburg.]

enzymes that can degrade cutin. Cutinases break cutin molecules and release monomers (single molecules) as well as oligomers (small groups of molecules) of the component fatty acid derivatives from the insoluble cutin polymer.

Fungi that penetrate the cuticle directly seem to constantly produce low levels of cutinase, which on contact with cutin releases small amounts of monomers. These subsequently enter the pathogen cell, trigger further expression of the cutinase genes, and stimulate the fungus to produce almost a thousand times more cutinase than before (Fig. 5-5). Cutinase production by the pathogen, however, may also be stimulated by some of the fatty acids present in the wax normally associated with cutin in the plant cuticle. However, the presence of glucose suppresses expression of the cutinase gene and reduces cutinase production drastically.

The involvement of cutinase in the penetration of the host cuticle by plant pathogenic fungi is shown by several facts. For example, the enzyme reaches its highest concentration at the penetrating point of the germ tube and at the infection peg of appressorium-forming fungi. Inhibition of cutinase by specific chemical inhibitors, or by antibodies of the enzyme applied to the plant surface, protects the plant from infection by fungal pathogens. Also, cutinase-deficient mutants show reduced virulence but become fully virulent when cutinase is added on the plant surface. In the brown rot of stone fruits, caused by the fungus *Monilinia fructicola*, fungal cutinase activity seems to be inhibited greatly by

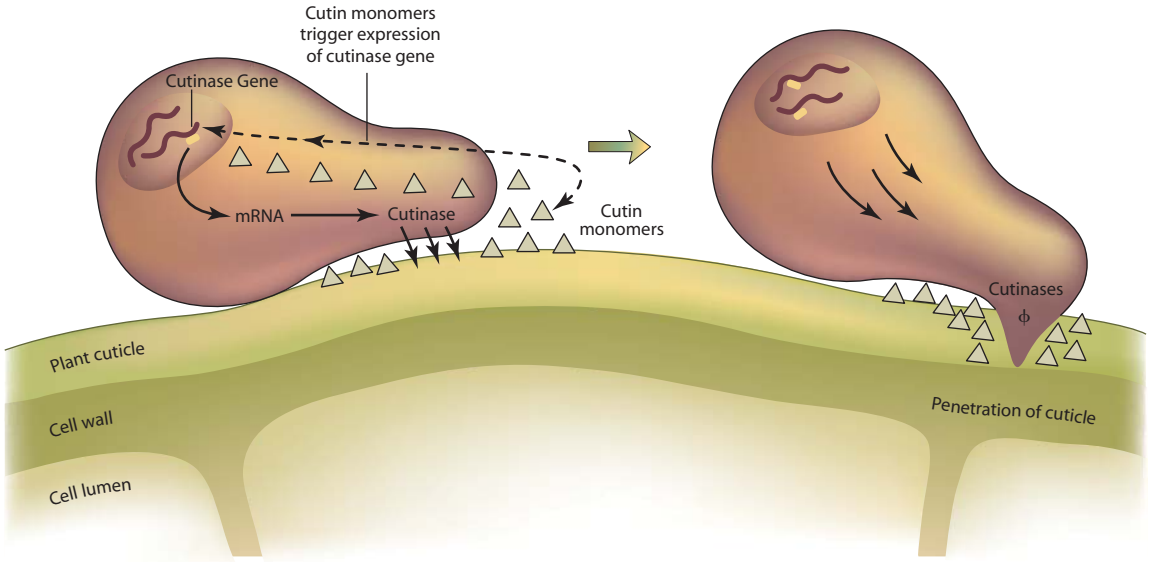


FIGURE 5-5 Diagrammatic representation of cuticle penetration by a germinating fungus spore. Constitutive cutinase releases a few cutin monomers from the plant cuticle. These trigger expression of the cutinase genes of the fungus, leading to the production of more cutinase(s), which macerates the cuticle and allows penetration by the fungus.

phenolic compounds such as chlorogenic and caffeic acids, which are abundant in epidermal cells of young fruit and the fruit is resistant to infection. As the fruit matures, the concentration of these compounds declines sharply, cutinase activity increases, and the fruit is penetrated by the fungus. Moreover, fungi that infect only through wounds and do not produce cutinase acquire the ability to infect directly if a cutinase gene from another fungus is introduced into them and enables them to produce cutinase. Pathogens that produce higher levels of cutinase seem to be more virulent than others. At least one study has shown that the germinating spores of a virulent isolate of the fungus *Fusarium* produced much more cutinase than those of an avirulent isolate of the same fungus and that the avirulent isolate could be turned into a virulent one if purified cutinase was added to its spores. The fungus *Botrytis cinerea*, the cause of numerous types of diseases on many plants, produces a cutinase and a lipase, both of which break down cutin. In the presence of antilipase antibodies, fungal spores failed to penetrate the cuticle and lesion formation was inhibited, indicating that lipase activity is required in at least the early stages of host infection.

Pectic Substances

Pectic substances constitute the main components of the middle lamella, i.e., the intercellular cement that holds in place the cells of plant tissues (Fig. 5-6). Pectic substances also make up a large portion of the primary cell wall in which they form an amorphous gel filling the spaces between the cellulose microfibrils (Fig. 5-7).

Pectic substances are polysaccharides consisting mostly of chains of galacturonan molecules interspersed with a much smaller number of rhamnose molecules and small side chains of galacturonan, xylan, and some other five carbon sugars. Several enzymes degrade pectic substances and are known as **pectinases** or **pectolytic enzymes** (Fig. 5-8). Some of them, e.g., the **pectin methyl esterases**, remove small branches off the pectin chains. Pectin methyl esterases have no effect on the overall chain length, but they alter the solubility of the pectins and affect the rate at which they can be attacked by the chain-splitting pectinases. The latter cleave the pectic chain and release shorter chain portions containing one or a few molecules of galacturonan. Some chain-splitting pectinases, called **polygalacturonases**, split the pectic chain by adding a molecule of water and breaking (hydrolyzing) the linkage between two galacturonan molecules; others, known as **pectin lyases**, split the chain by removing a molecule of water from the linkage, thereby breaking it and releasing products with an unsaturated double bond (Fig. 5-8). Polygalacturonases and pectin lyases occur in types that either can break the pectin chain at random sites (**endopectinases**) and release shorter chains, or can break only the terminal linkage (**exopectinases**) of the chain and release single units of galacturonan. The rhamnose and other sugars that may be forming part or branches of the pectin chain

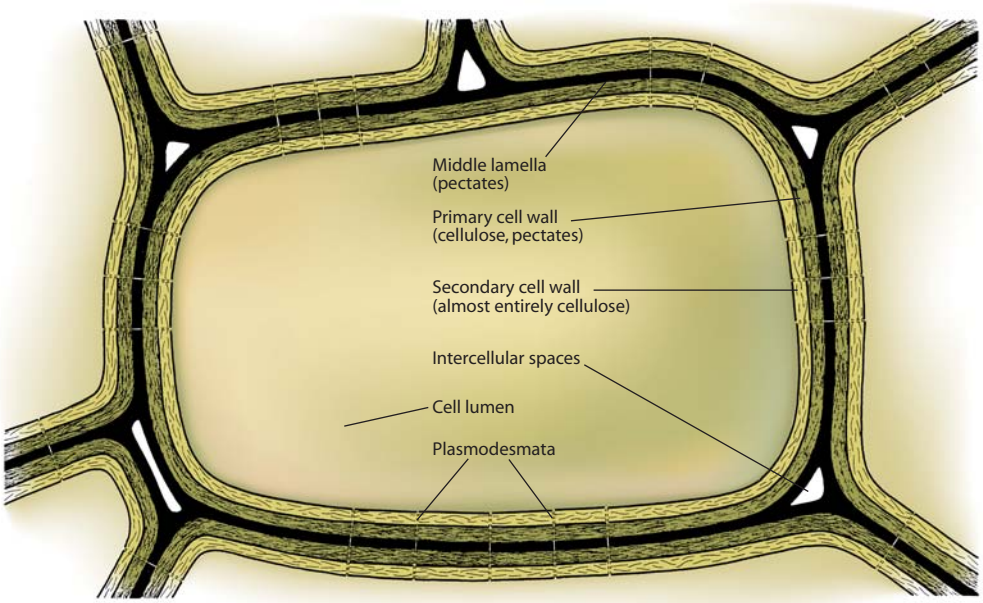


FIGURE 5-6 Schematic representation of the structure and composition of plant cell walls.

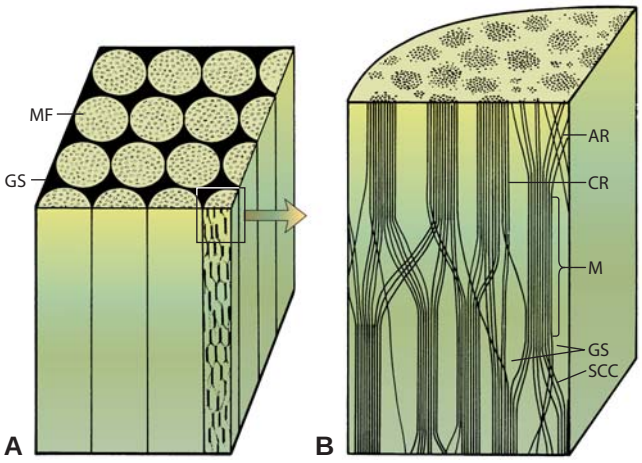


FIGURE 5-7 Schematic diagram of the gross struture of cellulose and microfibrils (A) and of the arrangement of cellulose molecules within a microfibril (B). MF, microfibril; GS, ground substance (pectin, hemicelluloses, or lignin); AR, amorphous region of cellulose; CR, crystalline region; M, micelle; SCC, single cellulose chain (molecule). [Adapted from Brown *et al.* (1949).]

are hydrolyzed by other enzymes that recognize these molecules.

As with cutinases, and with other enzymes involved in the degradation of cell wall substances, the production of extracellular pectolytic enzymes by pathogens is regulated by the availability of the pectin polymer and the released galacturonan units. The pathogen seems to produce at all times small, constitutive, base-level amounts of pectolytic enzymes that, in the presence of pectin, release from it a small number of galacturonan monomers, dimers, or oligomers. These molecules, when absorbed by the pathogen, serve as inducers for the enhanced synthesis and release of pectolytic enzymes (substrate induction), which further increase the amount of galacturonan monomers, etc. The latter are assimilated readily by the pathogen, but at higher concentrations they act to repress the synthesis of the same enzymes (catabolite repression), thus reducing production of the enzymes and the subsequent release of galacturonan monomers. The production of pectolytic enzymes is also repressed when the pathogen is grown in the presence of glucose. However, in some resistant host–pathogen combinations, pectolytic enzymes seem to elicit the plant defense response through the release from the cell wall of pectic fragments that function as endogenous elicitors of the defense mechanisms of the host.

Pectin-degrading enzymes have been shown to be involved in the production of many fungal and bacterial diseases, particularly those characterized by the soft rotting of tissues. Various pathogens produce different sets of pectinases and their isozymes. In some diseases, e.g., the bacterial wilt of solanaceous crops caused by *Ralstonia solanacearum*, pectinolytic enzymes collectively are absolutely essential for disease to develop, although some of them individually seem to not be required for disease but rather for accelerated colonization and enhanced aggressiveness by bacteria. In black rot of cantaloupe caused by the fungus *Didymella bryoniae*, there is a highly positive correlation between the

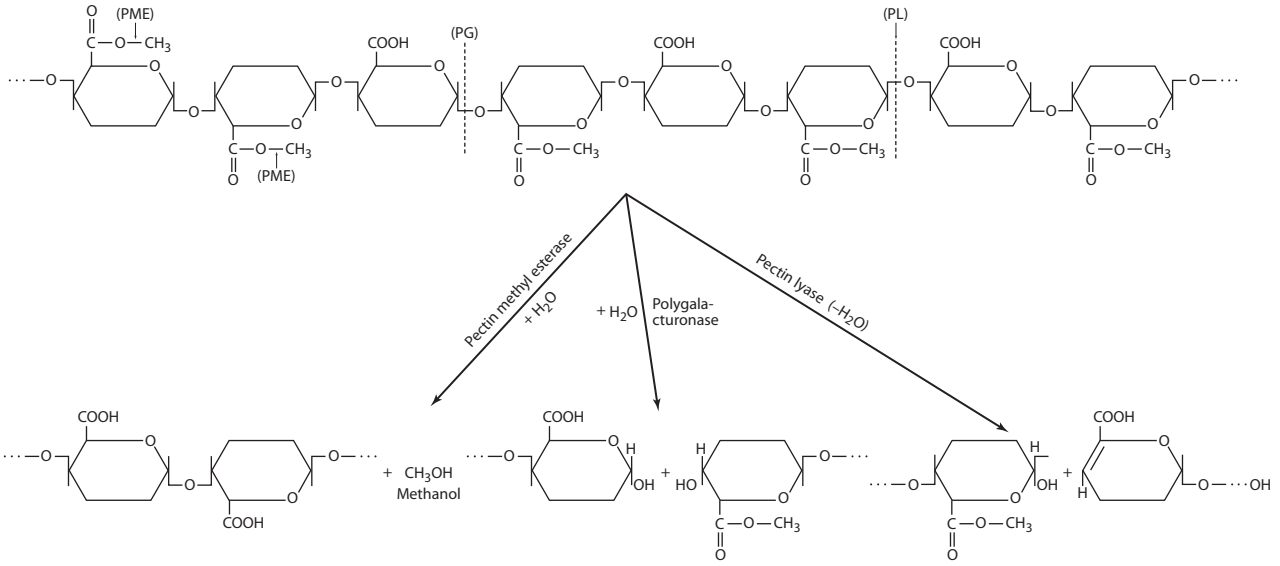


FIGURE 5-8 Degradation of a pectin chain by the three types of pectinases into modified and smaller molecules.

size of the rotting tissue lesion and the total fungal polygalacturonase activity in the rotting tissue.

In some *Colletotrichum*-caused anthracnoses, the fungus produces one pectin lyase that is a key virulence factor in disease development. The amount and activity of the enzyme and the amount of disease increase as the pH at the infection site increase to 7.5–8.0. The fungus maintains the high pH at the infection area by secreting ammonia. Inoculation of nonhost species in the presence of ammonia-releasing compounds enhances pathogenicity to levels similar to those caused by the compatible fungal and host species. Ammonia secretion by the fungus is a virulence factor for the fungus. Pectin–degrading enzymes are produced and play a role in the ability of nematodes, such as the root knot nematode, *Meloidogyne javanica*, for the penetration of root tissues, movement between plant cells along the middle lamella, and possibly in the formation of tee multinucleate giant cells on which the nematode feeds throughout the rest of its life. Some of these enzymes seem to affect the virulence of the pathogen on different hosts, i.e., they affect the degree of host specialization of the pathogen. Pectic enzymes are produced by germinating spores and, apparently, acting together with other pathogen enzymes (cutinases and cellulases), assist in the penetration of the host.

Pectin degradation results in liquefaction of the pectic substances that hold plant cells together and in the weakening of cell walls. This leads to tissue maceration, i.e., softening and loss of coherence of plant tissues and separation of individual cells, which eventually die (Fig. 5-9). The weakening of cell walls and tissue maceration undoubtedly facilitate the inter- or intracellular invasion of the tissues by the pathogen. Pectic enzymes also provide nutrients for the pathogen in infected tissues. Pectic enzymes, by the debris they create, seem to be involved in the induction of vascular plugs and occlusions in the vascular wilt diseases (Fig. 5-11). Although cells are usually killed quickly in tissues macerated by pectic enzymes, how these enzymes kill cells is not yet clear. It is thought that cell death results from the weakening by the pectolytic enzymes of the primary cell wall, which then cannot support the osmotically fragile protoplast, and the protoplast bursts.

Cellulose

Cellulose is also a polysaccharide, but it consists of chains of glucose (1–4) β-D-glucan molecules. The glucose chains are held to one another by a large number of hydrogen bonds. Cellulose occurs in all higher plants as the skeletal substance of cell walls in the form of microfibrils (see Figs. 5-7, 5-10, and 5-12). Microfibrils, which can be perceived as bundles of iron bars in a reinforced concrete building, are the basic structural units (matrix) of the wall, even though they account for less than 20% of the wall volume in most meristematic cells. The cellulose content of tissues varies from about 12% in the nonwoody tissues of grasses to about 50% in mature wood tissues to more than 90% in cotton fibers. The spaces between microfibrils and between micelles or cellulose chains within the microfibrils may be filled with pectins and hemicelluloses and probably some lignin at maturation. Although the bulk of cell wall polysaccharides is broken down by numerous enzymes produced by fungi and bacteria, a portion of them

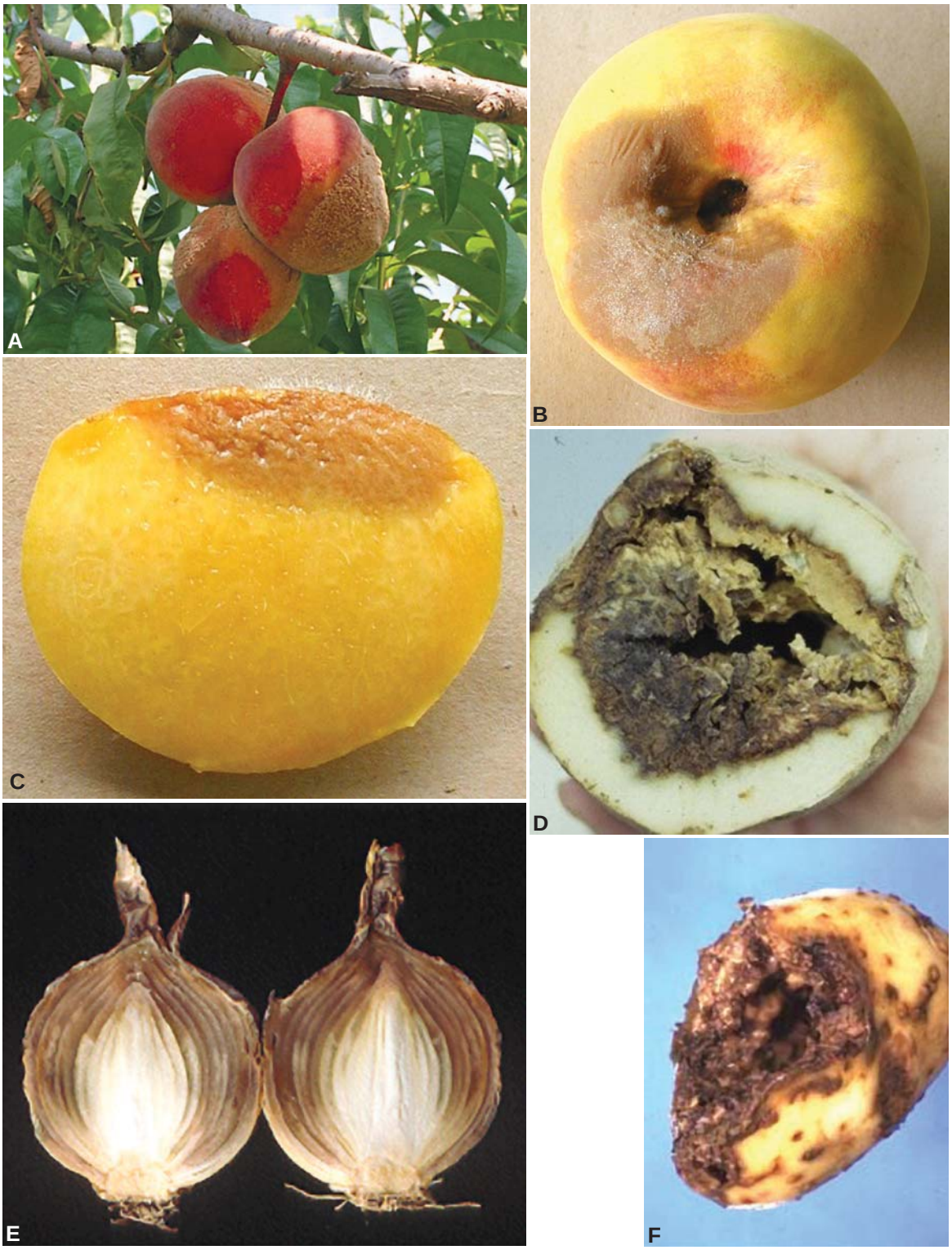


FIGURE 5-9 Involvement of pectolytic enzymes in disease development. Peach tissues infected with the brown rot fungus *Monilinia fructicola* while still on the tree (A) and by *Rhizopus* sp. at harvest (B and C) are macerated by the pectinases of the fungus and subsequently turn brown due to the oxidation of phenolic compounds released during maceration. Subsequent loss of water results in shrinking of the fruit. (D) Potato tuber, part of which has been macerated by the enzymes of the fungus *Fusarium* and subsequently has lost some of the water. An onion bulb (E) and a potato tuber (F) macerated by the enzymes of the fungus *Botrytis* and the bacterium *Erwinia*, respectively. [Photographs courtesy of (A) D. Ritchie, North Carolina State University, (D) P. Hamm, Oregon State University, (E) K. Mohan, University of Idaho, and (F) R. Rowe, Ohio State University.]

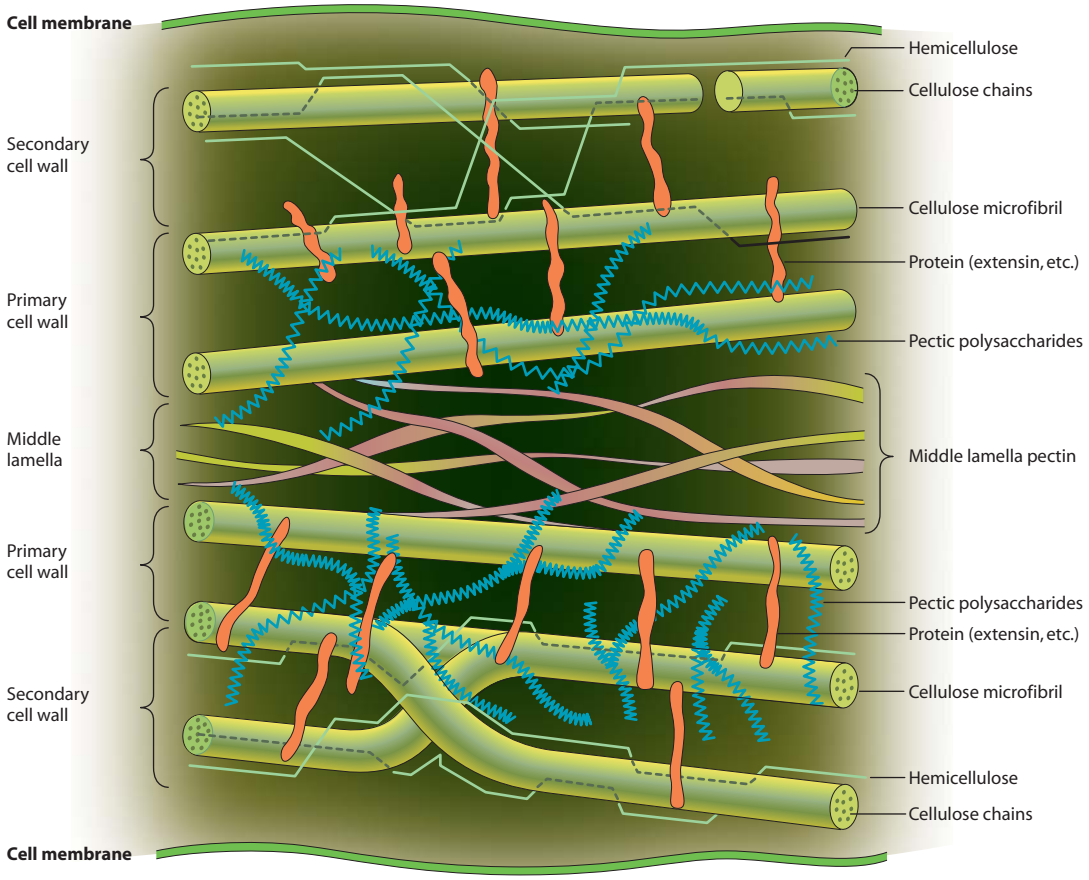


FIGURE 5-10 Schematic diagram of morphology and arrangement of some cell wall components.

appears to be broken down by nonenzymatic oxidative systems, such as activated oxygen and hydroxyl radicals (OH) produced during plant–fungus interactions. **Callose** differs from cellulose in that it consists of (1–3) β-D-glucan chains that can form duplexes and triplexes. Callose is normally made by a few cell types but is made by most cells following wounding and during attempted penetration by invading fungal hyphae.

The enzymatic breakdown of cellulose results in the final production of glucose molecules. The glucose is produced by a series of enzymatic reactions carried out by several cellulases and other enzymes. One cellulase (C1) attacks native cellulose by cleaving cross-linkages between chains. A second cellulase (C2) also attacks native cellulose and breaks it into shorter chains. These are then attacked by a third group of cellulases (Cx), which degrade them to the disaccharide cellobiose. Finally, cellobiose is degraded by the enzyme β-glucosidase into glucose.

Cellulose-degrading enzymes (cellulases) have been shown to be produced by several phytopathogenic fungi, bacteria, and nematodes and are undoubtedly produced by parasitic higher plants. Saprophytic fungi, mainly certain groups of basidiomycetes, and, to a lesser degree, saprophytic bacteria cause the breakdown of most of the cellulose decomposed in nature. In living plant tissues, however, cellulolytic enzymes secreted by pathogens play a role in the softening and disintegration of cell wall material (Figs. 5-11 and 5-12). They facilitate the penetration and spread of the pathogen in the host and cause the collapse and disintegration of the cellular structure, thereby aiding the pathogen in the production of disease. Cellulolytic enzymes may further participate indirectly in disease development by releasing, from cellulose chains, soluble sugars that serve as food for the pathogen and, in the vascular diseases, by liberating into the transpiration stream large molecules from cellulose, which interfere with the normal movement of water. In the bacterial wilt of tomato, production of an endocellulase by the bacterium was required for the latter to be pathogenic and induce the disease.

Cross-Linking Glycans (Hemicelluloses)

Cross-linking glycans, known earlier as hemicelluloses, are complex mixtures of polysaccharide

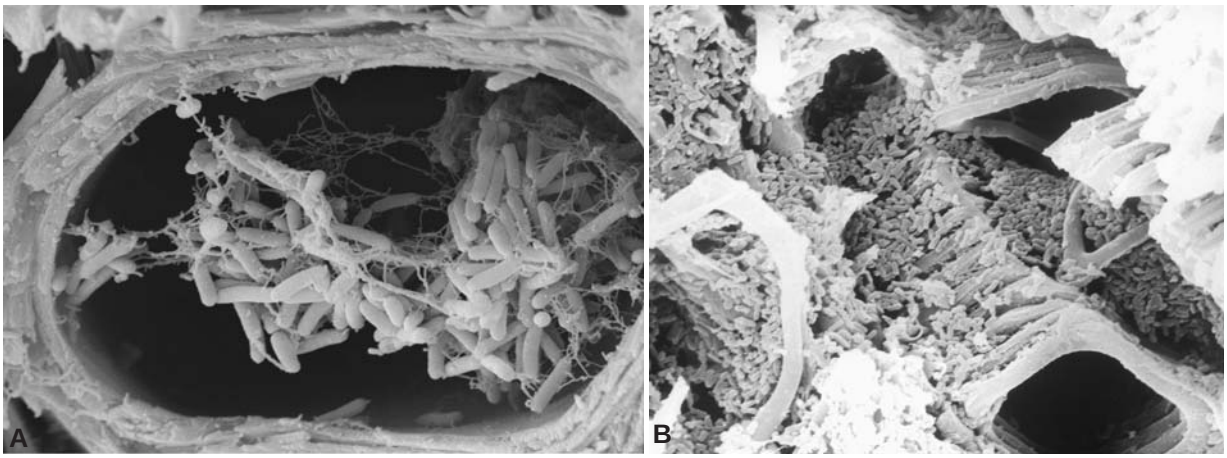


FIGURE 5-11 (A) Xylella bacteria in xylem vessel of citrus leaf. (B) Close-up of cell breakdown and maceration of pectic substances and celluloses of parenchyma cells and xylem vessels caused by enzymes secreted by bacteria of the genus *Pseudomonas*. Only the lignin-impregnated rings of xylem vessels remain intact. 1500×. [Photographs courtesy of (A) E. Alves, Federal University of Lavras, Brazil, and (B) E. L. Mansvelt, I. M. M. Roos, and M. J. Hattingh.]

polymers that can hydrogen-bond to and may cover and link cellulose microfibrils together (Figs. 5-10 and 5-12). Their composition and frequency seem to vary among plant tissues, plant species, and with the developmental stage of the plant. Cross-linking glycans are a major constituent of the primary cell wall and may also make up a varying proportion of the middle lamella and secondary cell wall. Hemicellulosic polymers include primarily xyloglucans and glucuronoarabinoxylans, but also glucomannans, galactomannans, arabinogalactans, and others. Xyloglucan, for example, is made of glucose chains with terminal branches of smaller xylose chains and lesser amounts of galactose, arabinose, and fucose. Cross-linking glycans link the ends of pectic polysaccharides and various points of the cellulose microfibrils.

The enzymatic breakdown of hemicelluloses appears to require the activity of many enzymes. Several hemicellulases seem to be produced by many plant pathogenic fungi. Depending on the monomer released from the polymer on which they act, the particular enzymes are called xylanase, galactanase, glucanase, arabinase, mannase, and so on. The nonenzymatic breakdown of hemicelluloses by activated oxygen, hydroxyl, and other radicals produced by attacking fungi also occurs. Despite the fact that fungal pathogens produce these enzymes and oxidative agents, it is still not clear how they contribute to cell wall breakdown or to the ability of the pathogen to cause disease.

Suberin

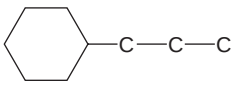
Suberin is found in certain tissues of various underground organs, such as roots, tubers, and stolons, and in periderm layers, such as cork and bark tissues. Suberins are also formed in response to wounding and to pathogen-induced defenses of certain organs and cell types. Typical suberization occurs, for example, in cut potato tubers where browning and encrustation develop in the form of multilamellar areas consisting of alternating polyaliphatic and polyaromatic layers. These layers are impermeable and help strengthen the cell wall and limit water loss through the wound. The aliphatic layer is composed of long chain (20 carbons or more) lipid substances, plus some specialized fatty acids, and is located between the primary cell wall and the plasmalemma. The polyaromatic layer consists of building blocks containing substances derived from hydroxycinnamic acid and is located in the cell wall. The polyaromatic layer also contains several phenolic compounds, such as chlorogenic acid, that act as local disinfectants. Although plants obviously produce enzymes that synthesize suberin, it is not known whether or how pathogens break it down during infection.

Lignin

Lignin is found in the middle lamella, as well as in the secondary cell wall of xylem vessels and the fibers that strengthen plants. It is also found in epidermal and occasionally hypodermal cell walls of some plants. The lignin content of mature woody plants varies from 15 to 38% and is second only to cellulose in abundance.

Lignin is an amorphous, three-dimensional polymer that is different from both carbohydrates and proteins in composition and properties. The most common basic structural unit of lignin is a phenylpropanoid:

FIGURE 5-12 (A and B) Cellulases, produced by the corn stalk rot fungus *Fusarium* sp., have broken down cellulosic walls of corn cells but did not affect the lignified vascular bundles. (C and D) Ligninases of the basidiomycete fungus *Phellinus* have caused complete disintegration and discoloration of the heartwood in the pine trunk (C) and of the roots and lower stem of the tree, causing it to topple over (D). [Photographs courtesy of (A and B) G. Munkvold, Iowa State University, (C) R. L. Anderson, USDA Forest Service, and (D) R. L. James, USDA Forest Service.]



where one or more of the carbons have a —OH, —OCH_3, or =O group. Lignin forms by oxidative condensation (C—C and C—O bond formation) between such substituted phenylpropanoid units. The lignin polymer is perhaps more resistant to enzymatic degradation than any other plant substance (Figs. 5-11 and 5-12).

It is obvious that enormous amounts of lignin are degraded by microorganisms in nature, as is evidenced by the yearly decomposition of all annual plants and a

large portion of perennial plants. It is generally accepted, however, that only a small group of microorganisms is capable of degrading lignin. Actually, only about 500 species of fungi, almost all of them basidiomycetes, have been reported so far as being capable of decomposing wood. About one-fourth of these fungi (the brown rot fungi) seem to cause some degradation of lignin but cannot utilize it. Most of the lignin in the world is degraded and utilized by a group of basidiomycetes called white rot fungi. It appears that white rot fungi secrete one or more enzymes (ligninases), which enable them to utilize lignin (Fig. 5-12).

In addition to wood-rotting basidiomycetes, several other pathogens, primarily several ascomycetes and imperfect fungi and even some bacteria, apparently produce small amounts of lignin-degrading enzymes and cause soft rot cavities in wood they colonize. However, it is not known to what extent the diseases they cause are dependent on the presence of such enzymes.

Cell Wall Flavonoids

Flavonoids are a large class of phenolic compounds that occur in most plant tissues and, especially, in the vacuoles. They also occur as mixtures of single and polymeric components in various barks and heartwoods. Among the various functions of flavonoids, some act as signaling molecules for certain functions in specific plant/microbe combinations. Many of them, however, are inhibitory or toxic to pathogens and some of them, e.g., medicarpin, act as phytoalexins and are involved in the inducible defense in plants against fungi. It is important, therefore, that pathogens be able to survive in the presence of various flavonoids in cell walls or they must be able to neutralize them or to break them down. Little is known how pathogens accomplish this, although the joining of phenolics with sugar molecules (glycosylation) seems to neutralize the toxicity of many phenolics.

Cell Wall Structural Proteins

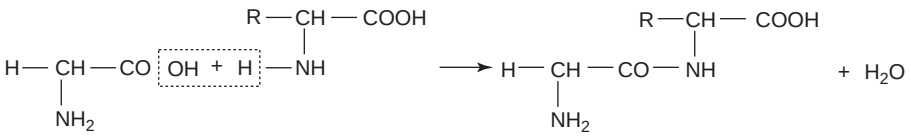
Cell walls consist primarily of polysaccharides, i.e., cellulose fibers embedded in a matrix of hemicellulose and pectin, but structural proteins, in the form of glycoproteins, may also form networks in the cell wall (Fig. 5-2). Four classes of structural proteins have been found in cell walls. Three of them are known by the most abundant amino acid they contain: **hydroxyproline-rich glycoproteins** (**HRGPs**), **proline-rich proteins** (**PRPs**), and **glycine-rich proteins** (**GRPs**). The fourth class is **arabinogalactan proteins** (**AGPs**). Each of these protein groups is coded by a large multigene family. Upon their production they are inserted in the endoplasmic reticulum and, through signal peptides they encode, they are targeted to the cell wall through the secretory pathway. One of the HRGP proteins is **extensin**, which makes up only 0.5% of the cell wall mass in healthy tissue but increases to 5 to 15% of the wall mass on infection with fungi and helps add rigidity to the cell wall. Another group of cell wall proteins are the **lectins**, which bind to specific sugar molecules**.** The role of all of these groups of proteins is not clear, but they are thought to accumulate in response to elicitor molecules released by fungi and to play a role in the plant defense response. The breakdown of structural proteins is presumably advantageous to invading pathogens and is thought to be similar to that of proteins contained within plant cells. This is discussed later.

Enzymatic Degradation of Substances Contained in Plant Cells

Most kinds of pathogens live all or part of their lives in association with or inside the living protoplast. These pathogens obviously derive nutrients from the protoplast. All the other pathogens — the great majority of fungi and bacteria — obtain nutrients from protoplasts after the latter have been killed. Some of the nutrients, e.g., sugars and amino acids, are molecules sufficiently small to be absorbed by the pathogen directly. Some of the other plant cell constituents, however, such as starch, proteins, and fats, can be utilized only after degradation by enzymes secreted by the pathogen.

Proteins

Plant cells contain innumerable different proteins, which play diverse roles as catalysts of cellular reactions (enzymes) or as structural material (in membranes and cell walls). Proteins are formed by the joining together of numerous molecules of about 20 different kinds of amino acids:



Amino Acids and Protein

All pathogens seem to be capable of degrading many kinds of protein molecules. The plant pathogenic enzymes involved in protein degradation are similar to those present in higher plants and animals and are called **proteases** or **proteinases** or, occasionally, peptidases.

Considering the paramount importance of proteins as enzymes, constituents of cell membranes, and structural components of plant cell walls, the degradation of host proteins by proteolytic enzymes secreted by pathogens can profoundly affect the organization and function of the host cells. The nature and extent of such effects, however, have been investigated little so far and their significance in disease development is not known.

Starch

Starch is the main reserve polysaccharide found in plant cells. Starch is synthesized in the chloroplasts and, in nonphotosynthetic organs, in the amyloplasts. Starch is a glucose polymer and exists in two forms: amylose, an essentially linear molecule, and amylopectin, a highly branched molecule of various chain lengths.

Most pathogens utilize starch, and other reserve polysaccharides, in their metabolic activities. The degradation of starch is brought about by the action of enzymes called **amylases**. The end product of starch breakdown is glucose and it is used by the pathogens directly.

Lipids

Various types of lipids occur in all plant cells, with the most important being **phospholipids** and **glycolipids**, both of which, along with protein, are the main constituents of all plant cell membranes. The latter form a hydrophobic barrier that is critical to life by separating cells from their surroundings and keeping organelles such as chloroplasts and mitochondria intact and separate from the cytoplasm. **Oils** and **fats** are found in many cells, especially in seeds where they function as energy storage compounds; **wax lipids** are found on most aerial epidermal cells. The common characteristic of all lipids is that they contain fatty acids, which may be saturated or unsaturated.

Several fungi, bacteria, and nematodes are known to be capable of degrading lipids. Lipolytic enzymes, called **lipases**, **phospholipases**, and so on, hydrolyze liberation of the fatty acids from the lipid molecule. The fatty acids are presumably utilized by the pathogen directly. But Some of them, before or after hyperoxidation by plant lipoxygenases or active oxygen species, provide signal molecules for the development of plant defenses and also act as antimicrobial compounds that inhibit the pathogen directly.

Microbial Toxins in Plant Disease

Living plant cells are complex systems in which many interdependent biochemical reactions are taking place concurrently or in a well-defined succession. These reactions result in the intricate and well-organized processes essential for life. Disturbance of any of these metabolic reactions causes disruption of the physiological processes that sustain the plant and leads to the development of disease. Among the factors inducing such disturbances are substances that are produced by plant pathogenic microorganisms and are called toxins. Toxins act directly on living host protoplasts, seriously damaging or killing the cells of the plant. Some toxins act as general protoplasmic poisons and affect many species of plants representing different families. Others are toxic to only a few plant species or varieties and are completely harmless to others. Many toxins exist in multiple forms that have different potency.

Fungi and bacteria may produce toxins in infected plants as well as in culture medium. Toxins, however, are extremely poisonous substances and are effective in very low concentrations. Some are unstable or react quickly and are bound tightly to specific sites within the plant cell.

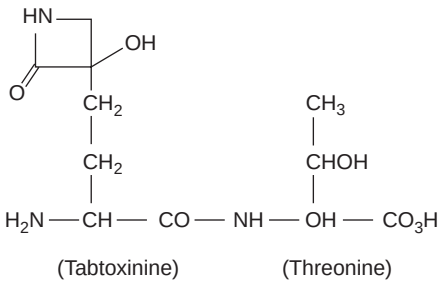
Toxins injure host cells either by affecting the permeability of the cell membrane (Fig. 5-2) or by inactivating or inhibiting enzymes and subsequently interrupting the corresponding enzymatic reactions. Certain toxins act as antimetabolites and induce a deficiency for an essential growth factor.

Toxins That Affect a Wide Range of Host Plants

Several toxic substances produced by phytopathogenic microorganisms have been shown to produce all or part of the disease syndrome not only on the host plant, but also on other species of plants that are not normally attacked by the pathogen in nature. Such toxins, called nonhost-specific or nonhost-selective toxins. These toxins increase the severity of disease caused by a pathogen, i.e., they affect the virulence of the pathogen, but are not essential for the pathogen to cause disease, i.e., they do not determine the pathogenicity of the pathogen. Several of these toxins, e.g., tabtoxin and phaseolotoxin, inhibit normal host enzymes, thereby leading to increases in toxic substrates or to depletion of needed compounds. Several toxins affect the cellular transport system, especially H^+/K^+ exchange at the cell membrane. Some, e.g., tagetitoxin, act as inhibitors of transcription in cell organelles, such as the chloroplasts. Others, e.g., cercosporin, act as photosensitizing agents, causing the peroxidation of membrane lipids.

Tabtoxin

Tabtoxin is produced by the bacterium *Pseudomonas syringae*; pv. *tabaci*, which causes the wildfire disease of tobacco; by strains of pv. tabaci occurring on other hosts such as bean and soybean; and by other pathovars (subspecies) of *P. syringae*, such as those occurring on oats, maize, and coffee. Toxin-producing strains cause necrotic spots on leaves, with each spot surrounded by a yellow halo (Figs. 5-13A and 5-13B). Sterile culture filtrates of the organism, as well as purified toxin, produce symptoms identical to those characteristic of wildfire of tobacco not only on tobacco, but in a large number of plant species belonging to many different families (nonhost-specific toxin!). Strains of *P. syringae* pv. *tabaci* sometimes produce mutants that have lost the ability to produce the toxin (they become Tox^-). Tox^- strains show reduced virulence and cause necrotic leaf spots without the yellow halo. Tox^- strains are indistinguishable from *P. angulata*, the cause of angular leaf spot of tobacco, which is now thought to be a nontoxigenic form of *P. syringae* pv. *tabaci*.



Tabtoxin

Tabtoxin is a dipeptide composed of the common amino acid threonine and the previously unknown amino acid tabtoxinine. Tabtoxin as such is not toxic, but in the cell it becomes hydrolyzed and releases tabtoxinine, which is the active toxin. Tabtoxin, through tabtoxinine, is toxic to cells because it inactivates the enzyme glutamine synthetase, which leads to depleted glutamine levels and, as a consequence, accumulation of toxic concentrations of ammonia. The latter uncouples photosynthesis and photorespiration and destroys the thylakoid membrane of the chloroplast, thereby causing chlorosis and eventually necrosis. The effects of the toxin lead to a reduced ability of the plant to respond actively to the bacterium.

Phaseolotoxin

Phaseolotoxin is produced by the bacterium *Pseudomonas syringae* pv. *phaseolicola*, the cause of halo blight of bean (Fig. 5-13C) and some other legumes. The localized and systemic chlorotic symptoms produced in infected plants are identical to those produced on plants treated with the toxin alone so they are apparently the results of the toxin produced by the bacteria. Infected plants and plants treated with purified toxin also show reduced growth of newly expanding leaves, disruption of apical dominance, and accumulation of the amino acid ornithine.

Phaseolotoxin is a modified ornithine–alanine–arginine tripeptide carrying a phosphosulfinyl group. Soon after the tripeptide is excreted by the bacterium into the plant, plant enzymes cleave the peptide bonds and release alanine, arginine, and phosphosulfinylornithine. The latter is the biologically functional moiety of phaseolotoxin. The toxin affects cells by binding to the active site of and inactivating the enzyme ornithine carbamoyltransferase, which normally converts ornithine to citrulline, a precursor of arginine. By its action on the enzyme, the toxin thus causes the accumulation of ornithine and depleted levels of citrulline and arginine. Phaseolotoxin, however, seems to also inhibit pyrimidine nucleotide biosynthesis, reduce the activity of ribosomes, interfere with lipid synthesis, change the permeability of membranes, and result in the accumulation of large starch grains in the chloroplasts. Phaseolotoxin plays a major role in the virulence of the pathogen by interfering with or breaking the disease resistance of the host toward not only the halo blight bacterium, but also several other fungal, bacterial, and viral pathogens.

Tentoxin

Tentoxin is produced by the fungus *Alternaria alternata* (previously called *A. tenuis*), which causes spots and chlorosis (Fig. 5-13D) in plants of many species. Seedlings with more than one-third of their leaf area chlorotic die, and those with less chlorosis are much less vigorous than healthy plants.

Tentoxin is a cyclic tetrapeptide that binds to and inactivates a protein (chloroplast-coupling factor) involved in energy transfer into chloroplasts. The toxin also inhibits the light-dependent phosphorylation of ADP to ATP. Both the inactivation of the protein and the inhibition of photophosphorylation are much greater in plant species susceptible to chlorosis after tentoxin treatment than in species not sensitive to the toxin. In sensitive species, tentoxin interferes with normal chloroplast development and results in chlorosis by disrupting chlorophyll synthesis, but it is not certain that these effects are solely related to tentoxin binding to the chloroplast-coupling factor protein. An additional but apparently unrelated effect of tentoxin on sensitive plants is that it inhibits the activity of polyphenol

FIGURE 5-13 Symptoms caused by nonhost-selective toxins. Early (A) and semiadvanced (B) symptoms of young tobacco leaves showing spots caused by the bacterium *Pseudomonas syringae* pv. *tabaci*. The chlorotic halos surrounding the necrotic white spots are caused by the tabtoxin produced by the bacterium. (C) Leaf spots and halos caused by the toxin phaseolotoxin produced by the bacterium *Pseudomonas phaseolicola*, the cause of halo blight of bean. (D) Leaf spots and chlorosis caused by the *Alternaria alternata* toxin. [Photographs courtesy of (A, B, and D) Reynolds Tobacco Co. and (C) Plant Pathology Department, University of Florida.]

oxidases, enzymes involved in several resistance mechanisms of plants. Both effects of the toxin, namely stressing the host plant with events that lead to chlorosis and suppressing host resistance mechanisms, tend to enhance the virulence of the pathogen. The molecular site of action of tentoxin, however, and the exact mechanism by which it brings about these effects are still unknown.

Cercosporin

Cercosporin is produced by the fungus *Cercospora* and by several other fungi. It causes damaging leaf spot and blight diseases of many crop plants, such as Cercospora leaf spot of zinnia (Fig. 5-14A) and gray leaf spot of corn (Fig. 5-14B).

Cercosporin is unique among fungal toxins in that it is activated by light and becomes toxic to plants by generating activated species of oxygen, particularly single oxygen. The generated active single oxygen destroys the membranes of host plants and provides nutrients for this intercellular pathogen. Cercosporin is a photosensitizing perylenequinone that absorbs light energy, it is converted to an energetically activated state and then reacts with molecular oxygen and forms activated oxygen. The latter reacts with lipids, proteins, and nucleic acids of plant cells and severely damages or kills the plant cells, thereby enhancing the virulence of the pathogen. The

FIGURE 5-14 Leaf spots on zinnia (A) and gray leaf spots on corn (B) caused by the photosensitizing toxin cercosporin, produced by different species of the fungus *Cercospora*. [Photographs courtesy of (A) Plant Pathology Department, University of Florida and (B) G. Munkvold, Iowa State University.]

ability of fungal spores and mycelium to survive the general toxicity of cercosporin is due to the production by the fungus of pyridoxine (vitamin B_6). Pyridoxine reacts with single oxygen atoms and is currently neutralized during that reaction.

Other Nonhost-Specific Toxins

Numerous other nonhost-specific toxic substances have been isolated from cultures of pathogenic fungi and bacteria and have been implicated as contributing factors in the development of the disease caused by the pathogen. Among such toxins produced by fungi are fumaric acid, produced by *Rhizopus* spp. in almond hull rot disease; oxalic acid, produced by *Sclerotium* and *Sclerotinia* spp. in various plants they infect and by *Cryphonectria parasitica*, the cause of chestnut blight; alternaric acid, alternariol, and zinniol produced by *Alternaria* spp. in leaf spot diseases of various plants; ceratoulmin, produced by *Ophiostoma ulmi* in Dutch elm disease; fusicoccin, produced by *Fusicoccum amygdali* in the twig blight disease of almond and peach trees; ophiobolins, produced by several *Cochliobolus* spp. in diseases of grain crops; pyricularin, produced by *Pyricularia grisea* in rice blast disease; fusaric acid and lycomarasmin, produced by *Fusarium oxysporum* in tomato wilt; and many others. Other nonhost-specific toxins produced by bacteria are coronatine, produced by *P. syringae* pv. *atropurpurea* and other forms infecting grasses and soybean; syringomycin, produced by *P. syringae* pv. *syringae* in leaf spots of many plants; syringotoxin, produced by *P. syringae* pv. *syringae* in citrus plants; and tagetitoxin, produced by *P. syringae* pv. *tagetis* in marigold leaf spot disease. One family of toxins essential for pathogenicity, is the thaxtomins, produced by species of the bacterium *Streptomyces* that cause root and tuber roots. Thaxtomins cause dramatic plant cell hypertrophy and/or seedling stunting by altering the development of primary cell walls and the ability of the cells to go through normal cell division cycles.

Host-Specific or Host-Selective Toxins

A **host-specific** or **host-selective** toxin is a substance produced by a pathogenic microorganism that, at physiological concentrations, is toxic only to the hosts of that pathogen and shows little or no toxicity against nonsusceptible plants. Most host-specific toxins must be present for the producing microorganism to be able to cause disease. So far, host-specific toxins have been shown to be produced only by certain fungi (*Cochliobolus*, *Alternaria*, *Periconia*, *Phyllosticta*, *Corynespora*, and *Hypoxylon*), although certain bacterial polysaccharides from *Pseudomonas* and *Xanthomonas* have been reported to be host specific.

Victorin, or HV Toxin

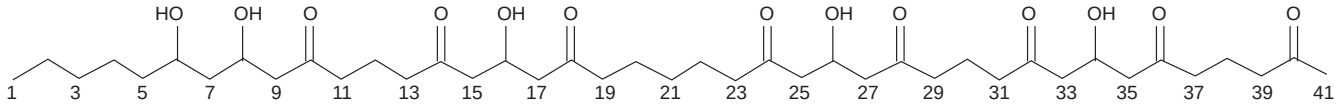
Victorin, or Hv-toxin, is produced by the fungus *Cochliobolus (Helminthosporium) victoriae*. This fungus appeared in 1945 after the introduction and widespread use of the oat variety Victoria and its derivatives, all of which contained the gene V_b for resistance to crown rust disease. *C. victoriae* infects the basal portions of susceptible oat plants and produces a toxin that is carried to the leaves, causes a leaf blight, and destroys the entire plant. All other oats and other plant species tested were either immune to the fungus and to the toxin or their sensitivity to the toxin was proportional to their susceptibility to the fungus. Toxin production in the fungus is controlled by a single gene. Resistance and susceptibility to the fungus, as well as tolerance and sensitivity to the toxin, are controlled by the same pair of alleles, although different sets of these alleles may be involved in cases of intermediate resistance. The toxin not only produces all the external symptoms of the disease induced by the pathogen, but it also produces similar histochemical and biochemical changes in the host, such as changes in cell wall structure, loss of electrolytes from cells, increased respiration, and decreased growth and protein synthesis. Moreover, only fungus isolates that produce the toxin in culture are pathogenic to oats, whereas those that do not produce toxin are nonpathogenic.

Victorin has been purified and its chemical structure has been determined to be a complex chlorinated, partially cyclic pentapeptide. The primary target of the toxin seems to be the cell plasma membrane where victorin seems to bind to several proteins. The possible site of action of victorin seems to be the glycine decarboxylate complex, which is a key component of the photorespiratory cycle. Considerable evidence, however, indicates that victorin functions as an elicitor that induces components of a resistance response that include many of the features of hypersensitive response and lead to programmed cell death.

*T Toxin [*Cochliobolus (Helminthosporium) heterostrophus *Race T Toxin]*

T toxin is produced by race T of *C. heterostrophus* (*Bipolaris maydis*), the cause of southern corn leaf blight (Fig. 5-15A). Race T, indistinguishable from all other *C. heterostrophus* races except for its ability to produce the T toxin, appeared in the United States in 1968. By 1970, it had spread throughout the corn belt, attacking only corn that had the Texas male-sterile (Tms) cytoplasm. Corn with normal cytoplasm was resistant to the fungus and the toxin. Resistance and susceptibility to *C. heterostrophus* T and its toxin are inherited maternally (in cytoplasmic genes). The ability of *C. heterostrophus* T to produce T toxin and its virulence to corn with Tms cytoplasm are controlled by one and the same gene. T toxin does not seem to be necessary for the pathogenicity of *C. heterostrophus* race T, but it increases the virulence of the pathogen.

T toxin is a mixture of linear, long (35 to 45 carbon) polyketols, the most prevalent having the following formula:



T toxin

The T toxin apparently acts specifically on mitochondria of susceptible cells, which are rendered nonfunctional, and inhibits ATP synthesis. The T toxin reacts with a specific receptor protein molecule (URF13) that is located on the inner mitochondrial membrane of sensitive mitochondria. It is now thought that plants exhibiting cytoplasmic male sterility of the Texas type have a slight rearrangement in their mitochondrial DNA comprising gene *T-urf13* that codes for the production of protein URF13. This gene and its protein are absent from maize lines with normal cytoplasm. When the T toxin is present, protein URF13 forms pores in the inner mitochondrial membrane of maize lines with cytoplasmic male sterility. The pores cause loss of mitochondrial integrity, i.e., loss of selective permeability of the mitochondrial membrane, and disease.

HC Toxin

Race 1 of *Cochliobolus (Helminthosporium) carbonum (Bipolaris zeicola)* causes northern leaf spot and ear rot disease in maize. It also produces the host-specific HC toxin, which is toxic only on specific maize lines. Two other races of the fungus do not produce toxin but infect corn around the world, although they cause smaller lesions. The mechanism of action of HC toxin is not known, but this is the only toxin, so far, for which the biochemical and molecular genetic basis of resistance against the toxin is understood. Resistant corn lines have a gene (Hm1) coding for an enzyme called HC toxin reductase that reduces and thereby detoxifies the toxin. Susceptible corn lines lack this gene and, therefore, cannot defend themselves against the

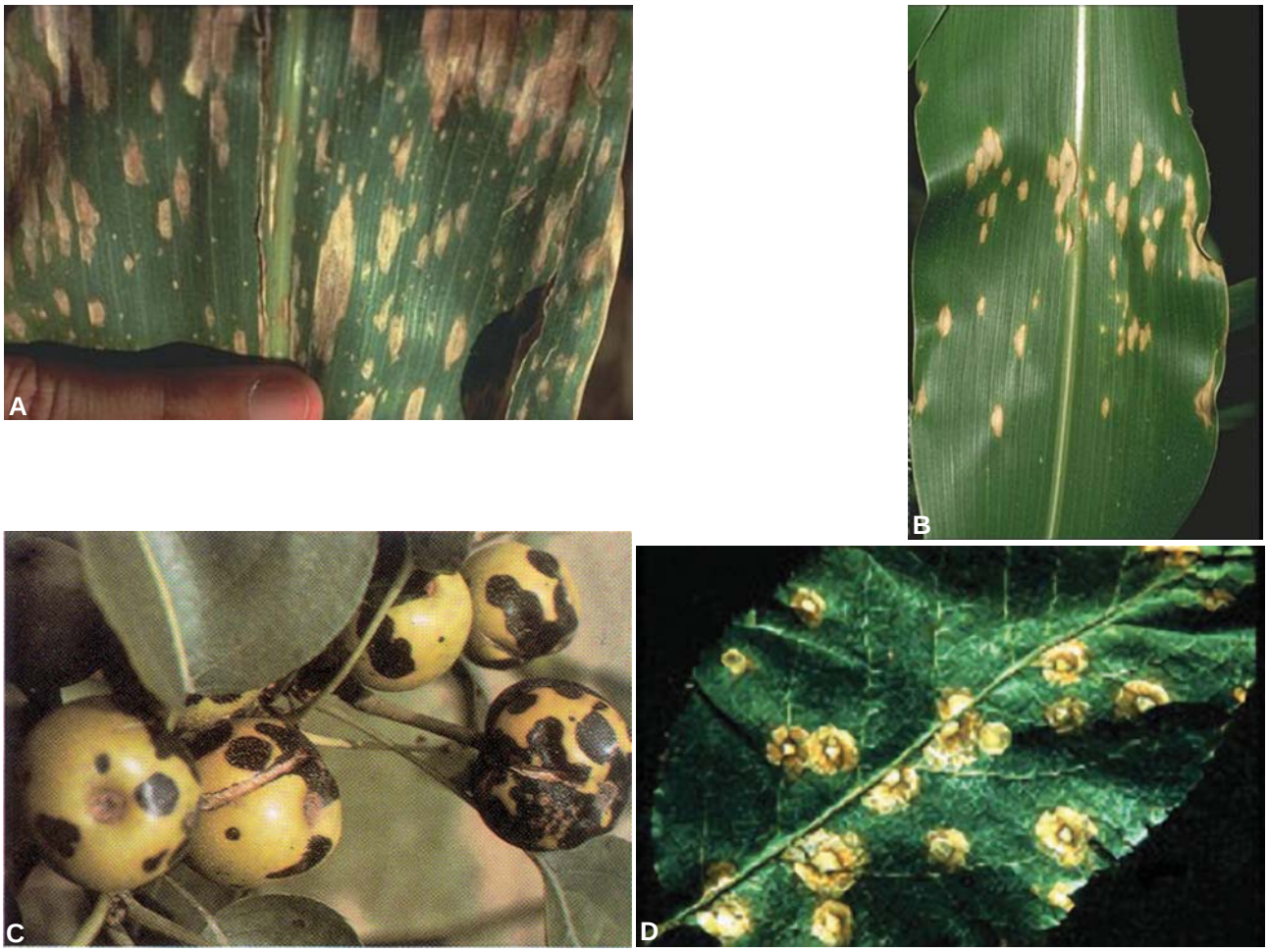


FIGURE 5-15 Symptoms caused by host-selective toxins. (A) Southern corn leaf blight symptoms caused by two race T of the fungus *Cochliobolus (Helminthosporium) heterostrophus* and its toxin, T toxin, on a corn plant containing Texas male-sterile cytoplasm. (B) Northern corn leaf spot symptoms caused by the fungus *Cochliobolus carbonum* and its toxin, HC toxin, on corn. (C) Fruit spots on Japanese pear caused by one of the strains of the fungus *Alternaria alternata* and its toxin, AK toxin. (D) Leaf spots caused by the AM toxin produced by another strain of the fungus *A. alternata* and its toxin, AM toxin, on apple leaves. [Photographs courtesy of (A) C. Martinson and (B) G. Munkvold, Iowa State University, (C) T. Sakuma, and (D) J. W. Travis, Pennsylvania State University.]

toxin. Experimental findings suggest that the HC toxin is not actually toxic in itself, but rather acts as a virulence factor by preventing initiation of the changes in gene expression that are necessary for the establishment of induced defense responses, i.e., it acts as a suppressor of defense responses.

Alternaria alternata *Toxins*

Several pathotypes of *Alternaria alternata* attack different host plants and on each they produce one of several multiple forms of related compounds that are toxic only on the particular host plant of each pathotype. Some of the toxins and the hosts on which they are produced and affect are the AK toxin causing black spot on Japanese peat fruit (Fig. 5-15C), the AAL toxin causing stem canker on tomato, the AF toxin on strawberry, the AM toxin on apple, the ACT toxin on tangerine, the ACL toxin on rough lemon, and the HS toxin on sugar cane.

As an example of *A. alternata* toxins, the AM toxin is produced by the apple pathotype of *A. alternata*, known previously as *A. mali*, the cause of alternaria leaf blotch of apple (Fig. 5-15D). The toxin molecule is a

cyclic depsipeptide and usually exists as a mixture of three forms. The toxin is extremely selective for susceptible apple varieties, whereas resistant varieties can tolerate more than 10,000 times as much toxin without showing symptoms. The AM toxin causes plasma membranes of susceptible cells to develop invaginations, and cells show a significant loss of electrolytes. The initial toxic effect of the toxin seems to occur at the interface between the cell wall and the plasma membrane. However, the AM toxin also causes rapid loss of chlorophyll, suggesting that this toxin has more than one site of action.

Other Host-Specific Toxins

At least two other fungi produce well-known host-specific toxins: *Periconia circinata* produces peritoxin (PC toxin), which causes sorghum rot in sorghum root rot disease; *Mycosphaerella (Phyllosticta) zeae-maydis* produces the PM toxin (T toxin) in corn that has Texas male-sterile cytoplasm; and *Pyrenophora tritici-repentis* produces the Ptr toxin, which causes the tan spot of wheat. Another fungus, *Corynespora cassiicola*, produces the CC toxin in tomato. Toxins produced by some other fungi, e.g., *Hypoxylon mammatum* on poplar and *Perenophora teres* on barley, seem to be species selective rather than host specific. In addition, there are the SV toxins produced by *Stemphylium vesicarium* on European pear and destruxin B from *A. brassicae* on brassicas.

Growth Regulators in Plant Disease

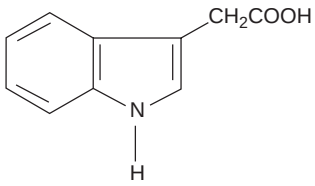
Plant growth is regulated by a small number of groups of naturally occurring compounds that act as hormones and are generally called growth regulators. The most important growth regulators are auxins, gibberellins, and cytokinins, but other compounds, such as ethylene and growth inhibitors, play important regulatory roles in the life of the plant. Growth regulators act in very small concentrations and even slight deviations from the normal concentration may bring about strikingly different plant growth patterns. The concentration of a specific growth regulator in the plant is not constant, but it usually rises quickly to a peak and then declines quickly as a result of the action of hormone-inhibitory systems present in the plant. Growth regulators appear to act, at least in some cases, by promoting the synthesis of messenger RNA molecules. This leads to the formation of specific enzymes, which in turn control the biochemistry and the physiology of the plant.

Plant pathogens may produce more of the same growth regulators as those produced by the plant or more of the same inhibitors of the growth regulators as those produced by the plant. They may produce new and different growth regulators or inhibitors of growth regulators. Alternatively, they may produce substances that stimulate or retard the production of growth regulators or growth inhibitors by the plant.

Whatever the mechanism of action involved, pathogens often cause an imbalance in the hormonal system of the plant and bring about abnormal growth responses incompatible with the healthy development of a plant. That pathogens can cause disease through the secretion of growth regulators in the infected plant or through their effects on the growth regulatory systems of the infected plant is made evident by the variety of abnormal plant growth responses they cause, such as stunting, overgrowths, rosetting, excessive root branching, stem malformation, leaf epinasty, defoliation, and suppression of bud growth. The most important groups of plant growth regulators, their function in the plant, and their role in disease development, where known, are discussed next.

Auxins

The auxin occurring naturally in plants is indole-3-acetic acid (IAA). Produced continually in growing plant tissues, IAA moves rapidly from the young green tissues to older tissues, but is destroyed constantly by the enzyme indole-3-acetic acid oxidase, which explains the low concentration of the auxin.



Indole-3-acetic acid

The effects of IAA on the plant are numerous. It is required for cell elongation and differentiation, and absorption of IAA to the cell membrane also affects the permeability of the membrane. The compound causes a general increase in the respiration of plant tissues and promotes the synthesis of messenger RNA and, subsequently, of proteins/enzymes as well as structural proteins.

Increased auxin (IAA) levels occur in many plants infected by fungi, bacteria, viruses, mollicutes, and nematodes, although some pathogens seem to lower the auxin level of the host. Thus, the basidiomycete *Exobasidium azaleae* causing azalea leaf and flower gall (Fig. 5-16A), the protozoon causing clubroot of cabbage (*Plasmodiophora brassicae*) (Fig. 5-16E), the bacterium

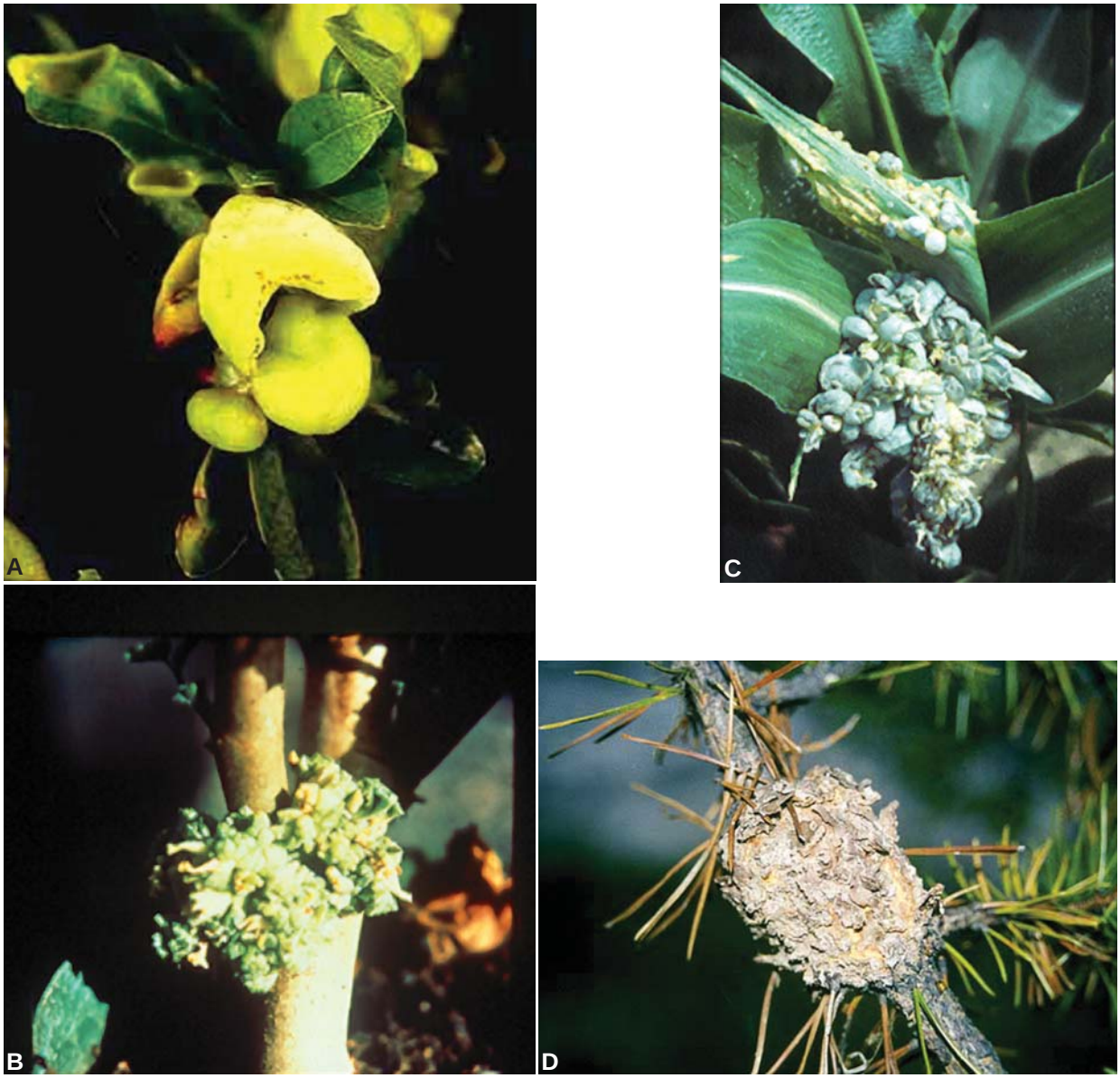


FIGURE 5-16 Plant diseases showing symptoms caused by the excessive production of growth regulators (primarily auxins) by the pathogen. (A) Enlarged and deformed leaf and flower gall of azalea caused by infection of the fungus *Exobasidium azaleae*. (B) Leafy gall produced on a sweet pea plant as a result of infection by the bacterium *Rhodococcus fascians*. (C) Corn ear and tassel showing numerous small galls as a result of infection by the corn smut fungus *Ustilago maydis*. (D) Western pine gall caused by the fungus *Cronartium* sp. (E) Cabbage roots enlarged grotesquely following infection with the clubroot pathogen *Plasmodiophora brassicae*. A few normal, thin roots are still present. (F) Root galls on bean plant infected with the root-knot nematode *Meloidogyne* sp. [Photographs courtesy of (A and B) Oregon State University, (C) K. Mohan, Idaho State University, (D) E. Hansen, Oregon State University, (E) University of Minnesota, and (F) R. T. MacMillan, University of Florida.]

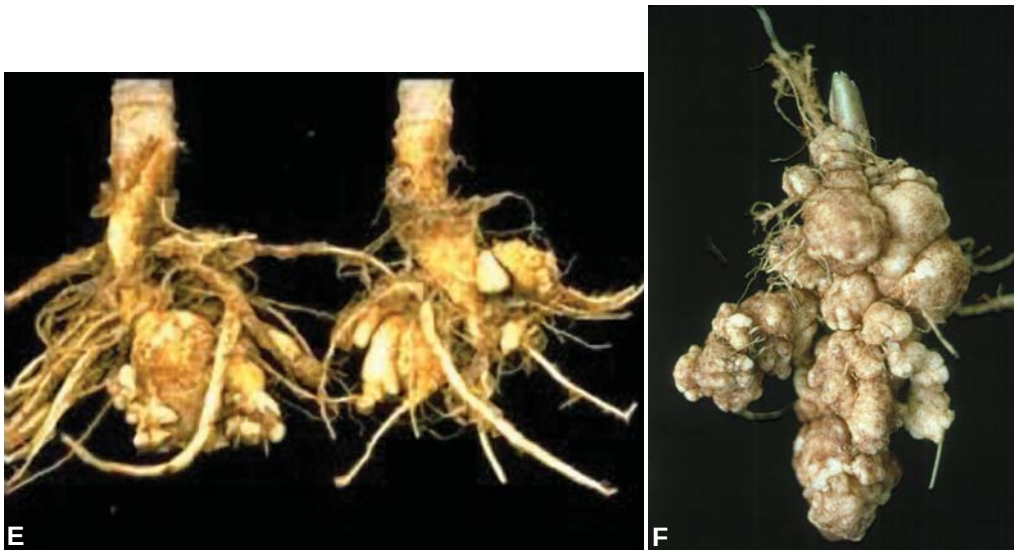


FIGURE 5-16 (*Continued*)

A. tumefaciens causing crown gall (Fig. 5-17A) and the one causing leafy gall of sweet pea and other plants (Fig. 5-16B), the fungi causing corn smut (*Ustilago maydis*) (Fig. 5-16C), cedar apple rust (*Gymnosporangium juniperi-virginianae*), banana wilt (*Fusarium oxysporum f. cubense*), pine western gall rust (Fig. 5-16D), the root-knot nematode (*Meloidogyne* sp.) (Fig. 5-16F), and others not only induce increased levels of IAA in their respective hosts, but are themselves capable of producing IAA. In some diseases, however, increased levels of IAA are wholly or partly due to the decreased degradation of IAA through the inhibition of IAA oxidase, as has been shown to be the case in several diseases, including corn smut and stem rust of wheat.

The production and role of auxin in plant disease have been studied more extensively in some bacterial diseases of plants. *Ralstonia solanacearum*, the cause of bacterial wilt of solanaceous plants, induces a 100-fold increase in the IAA level of diseased plants compared with that of healthy plants. How the increased levels of IAA contribute to the development of wilt of plants is not yet clear, but the increased plasticity of cell walls as a result of high IAA levels renders the pectin, cellulose, and protein components of the cell wall more accessible to, and may facilitate their degradation by, the respective enzymes secreted by the pathogen. An increase in IAA levels seems to inhibit the lignification of tissues and may thus prolong the period of exposure of the nonlignified tissues to the cell wall-degrading enzymes of the pathogen. Increased respiratory rates in the infected tissues may also be due to high IAA levels, and because auxin affects cell permeability, it may be responsible for the increased transpiration of the infected plants.

In **crown gall**, a disease caused by the bacterium *A. tumefaciens* on more than a hundred plant species, galls or tumors develop on the roots, stems (Figs. 3-2E, 3-11E, and 5-17A), leaves, ears, tassels, and petioles of host plants. Crown gall tumors develop when crown gall bacteria enter fresh wounds on a susceptible host. Immediately after wounding, cells around the wound produce various phenolic compounds and are activated to divide. *Agrobacterium* bacteria do not invade cells but attach to cell walls, and, in response to phenolic compounds such as acetosyringone and other signals, they become activated and begin processing the DNA in their Ti plasmid (for tumor-inducing plasmid) (Fig. 5-17). During the intense cell division of the second and third days after wounding, the plant cells are somehow conditioned and made receptive to a piece of bacterial plasmid DNA (called T-DNA, for tumor DNA). Proteins coded by genes in the T-DNA virulence (Vir) region cut out a single strand of the T-DNA from the Ti plasmid and transfer it into the plant cell nucleus as a T-DNA–protein complex. The T-DNA then becomes integrated into the nuclear plant DNA (chromosomes) and some of its genes are expressed and lead to the synthesis of auxins and cytokinins, which transform normal plant cells into tumor cells. Tumor cells subsequently grow and divide independently of the bacteria, and their

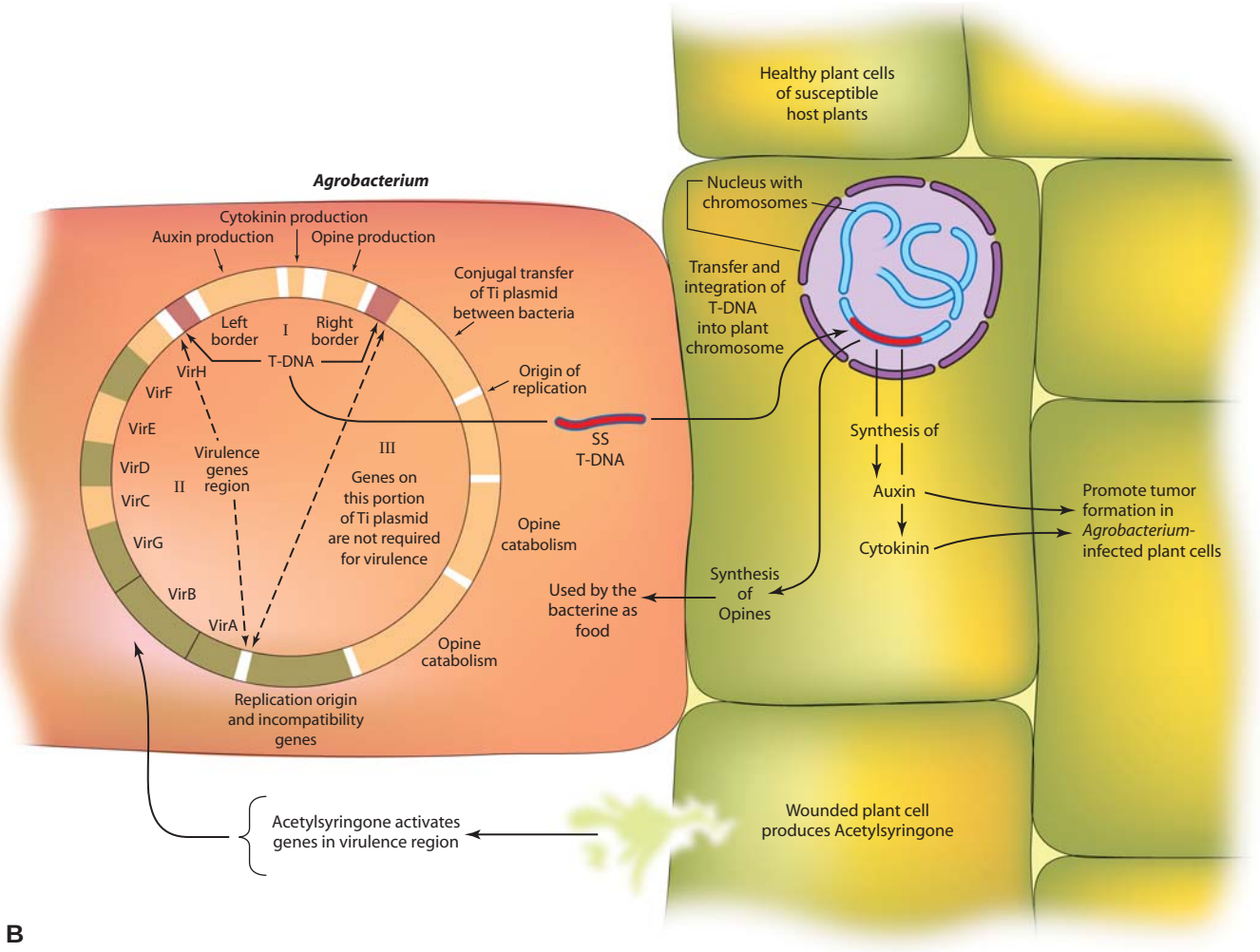


FIGURE 5-17 (A) External and cross-sectional view of crown gall on a rose stem caused by the bacterium *Agrobacterium tumefaciens*. (B) Schematic representation of the structure of Ti plasmid of the bacterium and of the transfer, integration, and expression of T-DNA in an infected plant that results in the production of crown gall tumors. Genes A, B, D, and G are needed for tumor formation on any susceptible plant species. Genes C, E, F, and H affect the host plant range and/or the size of tumors caused by the bacterium. The functions of the proteins of virulence genes are as follows: A, receptor of wound signal; B, codes for proteins that form membrane pores; C, enhances transfer of T-DNA; D, codes for proteins that nick T-DNA at its borders, help transport T-DNA across membranes, and carry signal compounds to the nucleus; E, protects T-DNA from nuclease enzymes and also carries nuclear localization signals; F, may increase host range of tumor induction; G, activates other virulence genes; H, protects the bacterium from toxic plant compounds. The entire diagram presents a simplified scheme of interaction of gene products of host cells and T-DNA that lead to the production of a gall. [Photograph (A) courtesy of Oregon State University.]

organization, rate of growth, and rate of division can no longer be controlled by the host plant.

The integrated T-DNA also contains genes that code for substances known as opines. Transformed plant cells produce opines, which can be used only by the intercellularly growing crown gall bacteria as a source of food. Although the increased levels of IAA and cytokinins of tumor cells are sufficient to cause the autonomous enlargement and division of these cells once they have been transformed to tumor cells, high IAA and cytokinin levels alone cannot cause the transformation of healthy cells into tumor cells. What other conditions or substances are involved in the transformation of healthy cells into tumor cells is not known.

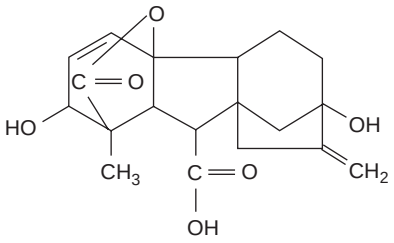
In the **knot disease** of olive, oleander, and privet, another hyperplastic disease caused by the bacterium *Pseudomonas savastanoi*, the pathogen produces IAA, which induces infected plants to produce galls. The more IAA a strain produces, the more severe the symptoms it causes. Strains that do not produce IAA fail to induce the formation of galls. The bacterial genes for IAA production are in a plasmid carried in the bacterium, but some IAA synthesis is also carried out by a gene in the chromosome of the bacterium.

In the **leafy gall disease** of many plants caused by the bacterium *Rhodococcus fascians*, leafy galls are produced that consist of centers of shoot overproductions and shoot growth inhibition. The bacterium exists mostly at the surface of the plant tissues, but it can also grow internally in the plant. Auxin, cytokinins, and other hormonal substances are produced by the bacterium in cultured and by infected tissues. Signals from bacteria involved in the development of symptoms initiate new cell divisions and formation of shoot meristem in tissues already differentiated. The bacterial signals originate in genes located on a linear plasmid and exert activities much more unique and more complex than those of cytokinins alone.

Gibberellins

Gibberellins are normal constituents of green plants and are also produced by several microorganisms. Gibberellins were first isolated from the fungus *Gibberella fujikuroi*, the cause of the foolish seedling disease of rice (Figure 1-37D). The best-known gibberellin is gibberellic acid. Compounds such as vitamin E and helminthosporol also have gibberellin-like activity.

Gibberellins have striking growth-promoting effects. They speed the elongation of dwarf varieties to normal sizes and promote flowering, stem and root elongation, and growth of fruit. Such *elongation* resembles in some respects that caused by IAA, and gibberellin also induces IAA formation. Auxin and gibberellin may also act synergistically. Gibberellins seem to activate genes that have been previously "turned off." The foolish seedling disease of rice, in which rice seedlings infected with the fungus *Gibberella fujikuroi* grow rapidly and become much taller than healthy plants, is apparently the result, to a considerable extent at least, of the gibberellin secreted by the pathogen.

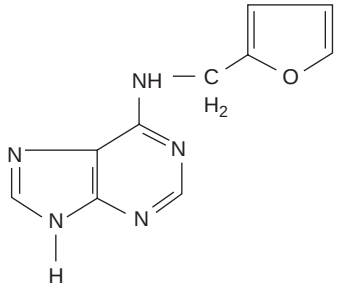


Gibberellins acid

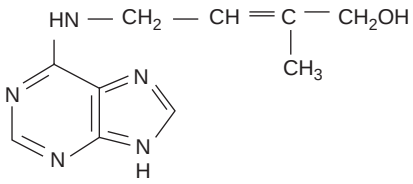
Although no difference has been reported so far in the gibberellin content of healthy and virus- or mollicute-infected plants, spraying of diseased plants with gibberellin overcomes some of the symptoms caused by these pathogens. Thus, stunting of corn plants infected with corn stunt spiroplasma and of tobacco plants infected with severe etch virus was reversed after treatment with gibberellin. Axillary bud suppression, caused by prunus dwarf virus (PDV) on cherry and by leaf curl virus on tobacco, was also overcome by gibberellin sprays. The same treatment also increased fruit production in PDV-infected cherries. In most of these treatments the pathogen itself does not seem to be affected and the symptoms reappear on the plants after gibberellin applications are stopped. It is not known, however, whether the pathogen-caused stunting of plants is actually due to reduced gibberellin concentration in the diseased plant, especially since the growth of even healthy plants is equally increased after gibberellin treatments.

Cytokinins

Cytokinins are potent growth factors necessary for cell growth and differentiation. In addition, they inhibit the breakdown of proteins and nucleic acids, thereby causing the inhibition of senescence, and they have the capacity to direct the flow of amino acids and other nutrients through the plant toward the point of high cytokinin concentration. Cytokinins occur in very small concentrations in green plants, in seeds, and in the sap stream. The first compound with cytokinin activity to be identified was kinetin, which, however, was isolated from herring sperm DNA and does not occur naturally in plants. Several cytokinins, e.g., zeatin and isopentenyl adenosine (IPA), have since been isolated from plants.



Kinetin



Zeatin

Cytokinins act by preventing genes from being turned off and by activating genes that have been previously turned off. The role of cytokinins in plant disease has just begun to be studied. Cytokinin activity increases in clubroot galls, in crown galls, in smut and rust galls, and in rust-infected bean leaves. In the latter, cytokinin activity seems to be related to both the juvenile feature of the green islands around the infection centers and the senescence outside the green island. However, cytokinin activity is lower in the sap and in tissue extracts of cotton plants infected with verticillium wilt and in plants suffering from drought. A cytokinin is partly responsible for several bacterial galls of plants, such as "leafy" gall disease of sweet pea caused by the bacterium *Rhodococcus (Corynebacterium) fascians*, and for the witches' broom diseases caused by fungi and mollicutes.

Treating plants with kinetin before or shortly after inoculation with a virus seems to reduce the number of infections in local lesion hosts and to reduce virus multiplication in systematically infected hosts.

Ethylene: $CH_2{=}CH_2$

Produced naturally by plants, ethylene exerts a variety of effects on plants, including chlorosis, leaf abscission, epinasty, stimulation of adventitious roots, and fruit ripening. Ethylene also causes increased permeability of cell membranes, which is a common effect of infections. However, ethylene production in infected tissues often parallels the formation of phytoalexins and the increased synthesis or activity of several enzymes or signal compounds that may play a role in increasing plant resistance to infection. Never-the-less it has not been shown that ethylene actually provides resistance. Ethylene is produced by several plant pathogenic fungi and bacteria. In the fruit of banana infected with *Ralstonia solanacearum*, the ethylene content increases proportionately with the (premature) yellowing of the fruit, whereas no ethylene can be detected in healthy fruits. Ethylene has also been implicated in the leaf epinasty symptom of the vascular wilt syndromes and in the premature defoliation observed in several types of plant diseases. In Verticillium wilt of tomato, the presence of ethylene at the time of infection inhibits disease development, whereas the presence of ethylene after infection has been established enhances Verticillium wilt development.

Polysaccharides

Fungi, bacteria, nematodes, and possibly other pathogens constantly release varying amounts of mucilaginous substances that coat their bodies and provide the interface between the outer surface of the microorganism and its environment. Exopolysaccharides appear to be necessary for several pathogens to cause normal disease symptoms either by being directly responsible for inducing symptoms or by indirectly facilitating pathogenesis by promoting colonization or by enhancing survival of the pathogen.

The role of slimy polysaccharides in plant disease appears to be particularly important in wilt diseases caused by pathogens that invade the vascular system of the plant. In vascular wilts, large polysaccharide molecules released by the pathogen in the xylem may be sufficient to cause a mechanical blockage of vascular bundles and thus initiate wilting (Figures 3-3E,F and 3-5D,E). Although such an effect by the polysaccharides alone may occur rarely in nature, when it is considered together with the effect caused by the macromolecular substances released in the vessels through the breakdown of host substances by pathogen enzymes, the possibility of polysaccharide involvement in the blockage of vessels during vascular wilts becomes obvious.

Detoxification of Low Molecular Weight Antimicrobial Molecules

Several kinds of low molecular weight antimicrobial molecules are present in plants or are produced by them

in response to infection by pathogens. Some of the most common constitutive such substances are the **saponins**, which include the avenacins and the tomatines. Saponins are glycosylated triterpenoid or steroid alkaloid molecules that provide plants with some degree of protection against fungal pathogens. Saponins are thought to provide antifungal protection by forming complexes with cell membranes, leading to the formation of pores and loss of membrane integrity.

Avenacins are produced in oat roots and leaves and they protect oats from the root-infecting fungus *Gaeumannomyces graminis* while it infects the other cereals that contain no avenacins. A strain of the fungus that infects oats, *G. graminis f. sp. avenae*, produces the avenacin-detoxifying enzyme avenacinase, which is required for pathogenicity on oats. Also, the fungus *Stagonospora avenae* can infect oat leaves, despite the fact that they contain avenacins, by secreting at least three enzymes that degrade and detoxify the avenacins. Another saponin, tomatine, is present in tomatoes, which are protected from infection by some fungi that lack the tomatinase enzyme needed for tomatine detoxification. The fungus *Septoria lycopersici* produces tomatinase and infects tomato plants. Mutants of this fungus, however, that do not produce tomatinase were sensitive to tomatine but could still grow in its presence. They could cause lesions on tomato leaves that actually had more dying mesophyll cells and greater activity of a defense-related enzyme. It is not clear whether this behavior of the host is the result of differences between the mutants and the normal strains or whether the production of tomatinase helps suppress some mechanism(s) of plant defense. In *Botrytis cinerea*, all but 1 of 13 isolates could detoxify tomatine and could severely infect tomato, while one strain that was more sensitive to tomatine was also much less aggressive on tomato.

Promotion of Bacterial Virulence by *avr* Genes

avr genes in bacteria are thought to encode or to direct the production of molecules that are recognized by the host plant and elicit the rapid induction of defense responses on resistant host plants. Their prevalence among pathogens, however, suggests that they may provide some advantage to the pathogen in addition to warning host plants that they are about to be attacked. It has been proposed, therefore, and been demonstrated in many plant–bacteria combinations, that the proteins (Avr proteins) coded for by *avr* genes promote pathogen growth and disease development in susceptible hosts. How Avr proteins accomplish that is not known, but they have been shown to interfere with the resistance mediated by the *avr* gene. Because the Avr proteins are coded for by the *avr* genes, it is apparent that *avr* genes can modify the signaling of host defense pathways in resistant hosts. In some cases, in the absence of a resistance R gene, the particular *avr* gene acts as a virulence factor that not only promotes growth of the particular bacterium in several hosts, including some that exhibit varying degrees of resistance, but transgenic plants that express the *avr* gene actually exhibit enhanced susceptibility to the pathogen and/or aggressiveness of the pathogen. Different *avr* genes, however, even of the same bacterial pathogen, contribute different degrees of susceptibility/aggressiveness to bacteria that provide these genes. This shows that the particular Avr proteins function inside the host plant cell and promote bacterial virulence.

Role of Type III Secretion in Bacterial Pathogenesis

Although the primary determinants of pathogenicity and virulence in many bacteria are secreted enzymes such as pectin lyases, cellulases, and proteases that macerate plant tissues of many species, it is now known that in at least *Erwinia* bacteria, the genes for hypersensitive reaction and pathogenicity (*hrp* genes) determine the potential secondary pathogenesis. In plant pathogens, *hrp* genes code for a type III secretion machinery, which is thought to transport bacterial effector proteins directly into the host cell. *hrp* genes exist in clusters of about 20 genes, one of which codes for a constituent of an outer membrane, whereas many others code for the core secretion machinery, for regulatory genes, for harpins, for the Hrp-pilin, which in some bacteria is required for type III secretion to function, for avirulence (*avr*) genes, and so on. In nonmacerating bacteria *Pseudomonas*, *Ralstonia*, and *Xanthomonas* and in the fire blight bacterium *Erwinia amylovora*, *hrp* genes are essential for virulence and elicitation of a hypersensitive response.

Suppressors of Plant Defense Responses

It has been shown that at least some plant pathogenic fungi, e.g., *Puccinia graminis* f. sp. *tritici*, which causes stem rust of wheat, and *Mycosphaerella pinodes*, which causes a leaf spot on pea, produce substances called **suppressors** that act as pathogenicity factors by suppressing the expression of defense responses in the host plant. The defense suppressor of the wheat stem rust fungus has been found in the fungus germination fluid and in the intercellular fluid of rust-infected wheat leaves. This suppressor interacts with the wheat cell

plasma membrane and reduces binding of the pathogen's 67-kDa glycoprotein elicitor of host defenses to the plasma membrane. In this way, the suppressor molecule suppresses the activity of phenylalanine lyase (PAL) and the normal development of defense responses. The pea-infecting fungus produces two suppressors in the spore germination fluid. Both suppressors are glycopeptides, counteract the elicitor of phytoalexin biosynthesis, and temporarily suppress the expression of all defense reactions of the host plant. The *Mycosphaerella* suppressors seem to reduce the proton-pumping activity of the host cell membrane ATPase and thereby temporarily lower the ability of the cell to function and to defend itself. A different mechanism of suppression of plant defense responses has been reported in the ergot disease of rye caused by the fungus *Claviceps purpurea*. In that disease the fungus produces the enzyme catalase, which reacts with and neutralizes the hydrogen peroxide that is produced as one of the first defense responses of plants against infecting pathogens. The fungal catalase concentration is greatest at hyphal walls and hyphal surfaces and is secreted by the fungus into the host apoplast at the host–pathogen interface, where the host H_2O_2 is produced. By inactivating active oxygen species produced by the host through catalase, the fungus suppresses the host defenses.

Pathogenicity and Virulence Factors in Viruses and Viroids

Until recently, little was known about the intrinsic factors of viruses and viroids that determine their pathogenicity and/or virulence. Viruses have a few, usually less than 10, genes, yet they are very capable pathogens. This requires that viral genes and gene products have multitask functions. Some of the most basic functions viral genes control are infectivity on a particular host, replication of the virus, movement of the virus from cell to cell, long-distance transport of the virus in the plant, transmissibility of the virus from plant to plant, and production of the coat protein of the virus. All of these functions are necessary for the pathogenicity and survival of the virus, although the variation in the degree most of these functions are carried out affects the virulence of the virus, i.e., the level of disease and symptoms it can cause in a host plant, rather than its pathogenicity, i.e., its ability to infect a plant.

Plant viruses have no genes that allow them to produce macerating enzymes, toxins, growth regulators, or other biologically active compounds by which to affect plant cells. However, different viruses manage to induce the plant to develop symptoms that appear to be the result of action and interaction of numerous such compounds present in the cell, despite the fact that no such compound can be found in infected cells. How viruses cause disease remains, therefore, pretty much a mystery but some facts are beginning to emerge.

One of the most important proteins coded by viruses that plays an important role in their pathogenicity and virulence is their coat protein. In addition to protecting the viral nucleic acid from external damaging factors, the coat protein plays important roles in practically everything pertaining to viral replication and dissemination. Thus, the coat protein plays a role in host recognition, uncoating and release of the nucleic acid, assistance in replication of the nucleic acid, movement of the virus between cells and organs, movement of the virus via a vector between plants, and modification of symptoms. Again, little is known on the mechanisms by which the coat protein affects these functions.

Another viral protein that has been studied extensively is the so-called movement protein, which enables viruses to move between cells and/or through the phloem system of the plant by altering the properties of plasmodesmata. However, some movement proteins not only open movement channels for the virus, they also block a defense molecule, the suppressor of virus silencing by the plant cell activated by the viral infection. Some viroids seem to form complexes with certain host proteins that help the viroids pass through plasmodesmata and with plant lectins that help viroids move through the phloem of host plants.

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Chapter six

HOW PLANTS DEFEND THEMSELVES AGAINST PATHOGENS

Each plant species is affected by approximately 100 different kinds of fungi, bacteria, mollicutes, viruses, and nematodes. Frequently, a single plant is attacked by hundreds, thousands, and, in leafspot diseases of large trees, probably hundreds of thousands of individuals of a single kind of pathogen. Although such plants may suffer damage to a lesser or greater extent, many survive all these attacks and, not uncommonly, manage to grow well and to produce appreciable yields.

In general, plants defend themselves against pathogens by a combination of weapons from two arsenals: (1) structural characteristics that act as physical barriers and inhibit the pathogen from gaining entrance and spreading through the plant and (2) biochemical reactions that take place in the cells and tissues of the plant and produce substances that are either toxic to the pathogen or create conditions that inhibit growth of the pathogen in the plant. The combinations of structural characteristics and biochemical reactions employed in the defense of plants are different in different host–pathogen systems. In addition, even within the same host and pathogen, the combinations vary with the age of the plant, the kind of plant organ and tissue attacked, the nutritional condition of the plant, and the weather conditions.

WHATEVER THE PLANT DEFENSE OR RESISTANCE, IT IS CONTROLLED BY ITS GENES

One concept that must be made clear at the outset is that whatever the kind of defense or resistance a host plant employs against a pathogen or against an abiotic agent, it is ultimately controlled, directly or indirectly, by the genetic material (genes) of the host plant and of the pathogen (Fig. 6-1).

Nonhost Resistance

A plant may find it easy to defend itself, i.e., to stay resistant (immune) when it is brought in contact with a pathogenic biotic agent to which the plant is not a host. This is known as nonhost resistance and is the most common form of resistance (or defense from attack) in nature. For example, apple trees are not affected by pathogens of tomato, of wheat, or of citrus trees because the genetic makeup of apple is in some way(s) different from that of any other kinds of host plants, which, of course, are attacked by their own pathogens. However, apple can be attacked by its own pathogens, which, in turn, do not attack tomato, wheat, citrus, or anything else. Similarly, the fungus that causes powdery mildew on wheat (*Blumeria graminis* f. sp. *tritici*) does not infect barley and vice versa, the fungus that causes powdery mildew on barley (*B. graminis* f. sp. *hordei*) does not infect wheat, and so on. All such unsuccessful plant/pathogen interactions are thought to represent nonhost resistance. It has been shown recently however, that in at least some related pairings, e.g., the wheat, powdery mildew fungus inoculated on barley, the fungus produces haustoria and the host reacts by producing hydrogen peroxide (H_2O_2), cell wall appositions under the appressoria, and a hypersensitive response in which epidermal cells die rapidly in response to fungal attack.

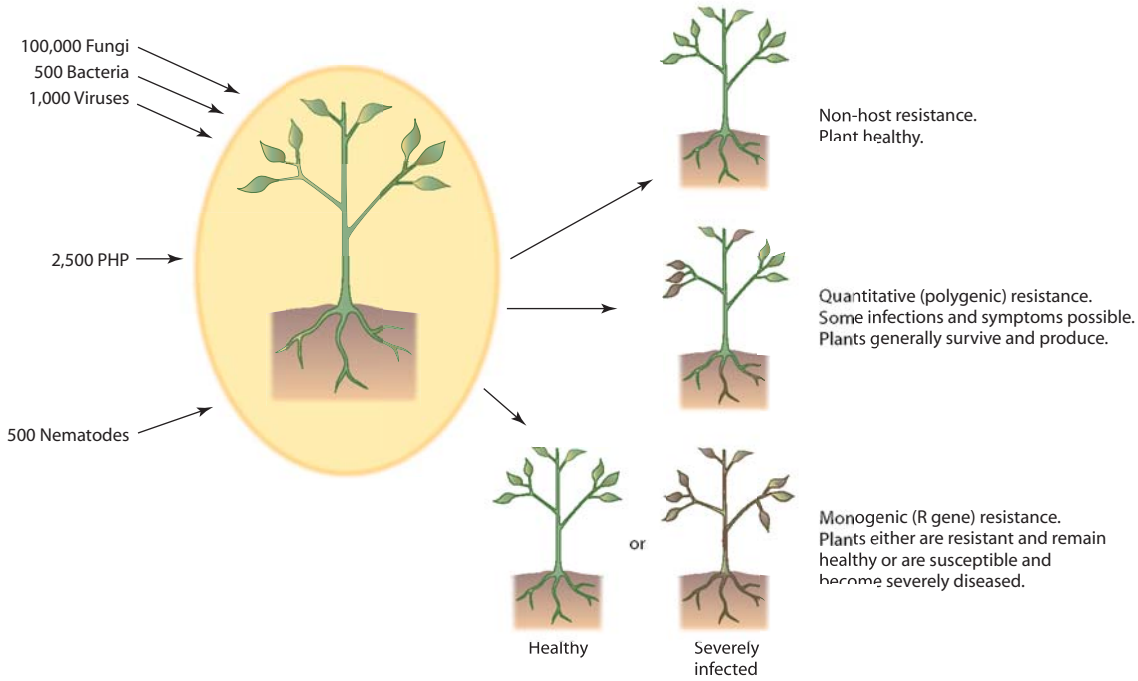


FIGURE 6-1 Types of reaction of plants to attacks by various pathogens in relation to the kind of resistance of the plant.

Partial, Polygenic, Quantitative, or Horizontal Resistance

Each plant, of course, is attacked by its own pathogens, but there is often a big difference in how effectively the plant can defend itself (how resistant the plant is) against each pathogen. Even when conditions for infection and disease development are favorable, a plant, upon infection with a particular pathogen, may develop no disease, only mild disease, or severe disease, depending on the specific genetic makeup of the plant and of the pathogen that attacks it. Many genes are involved in keeping a plant protected from attack by pathogens. Many of these genes provide for the general upkeep and well-being functions of plants, but plants also have many genes whose main functions seem to be the protection of plants from pathogens. Some of the latter plant genes code for chemical substances that are toxic to pathogens or neutralize the toxins of the pathogens, and these substances may be present in plants regardless of whether the plant is under attack or not. Plants also have genes that produce and regulate the formation of structures that can slow down or stop the advance of a pathogen into the host and cause disease. These structures can also be present in a plant throughout its life or they may be produced in response to attack by one of several pathogens or following injury by an abiotic agent. Preexisting defense structures or toxic chemical substances, and many of those formed in response to attack by a pathogen or abiotic agent, are important in the defense of most plants against most pathogens.

When a pathogen attacks a host plant, the genes of the pathogen are activated, produce, and release all their weapons of attack (enzymes, toxins, etc.) against the plants that they try to infect. With the help of different combinations of preexisting or induced toxic chemical substances or defense structures, most plants manage to defend themselves partially or nearly completely. Such plants show sufficient resistance that allows them to survive the pathogen attacks and to produce a satisfactory yield. This type of defense or resistance is known as polygenic, general, or quantitative resistance because it depends on many genes for the presence or formation of the various defense structures and for preexisting or induced production of many substances toxic to the pathogen. This type of resistance is present at different levels against different pathogens in absolutely all plants and is also known as partial, quantitative, horizontal, multigenic, field, durable, or minor gene resistance.

Most plants depend on general resistance against their pathogens, especially nonobligate parasites, e.g., the semibiotrophic or nectrotrophic oomycetes *Pythium* and *Phytophthora*, the fungi *Botrytis*, *Fusarium*, *Sclerotinia*, and *Rhizoctonia*, and most bacteria, nematodes, and so on. In at least some polygenic plant–pathogen combinations, such as the early blight of tomato caused by the

necrotrophic fungus *Alternaria solani*, the more resistant the varieties are, the higher the constitutive concentration and the more rapid the accumulation in them of pathogen-induced pathogenesis related (PR) proteins, than in susceptible varieties. These PR proteins include some of the specific antifungal isozymes of chitinase and β-1,3-glucanase. Also, total enzyme preparations from resistant varieties were able to release elicitors of the hypersensitive response (HR) (see later) from purified fungal cell walls, whereas enzymes from susceptible varieties could not. Furthermore, partially purified chitinases from tomato leaves could release HR elicitors from germinating *A. solani* spores but not from mature intact cell walls. This suggests that, perhaps, constitutively produced hydrolytic enzymes may act as a mechanism of elicitor release in tomato resistance to the early blight disease. Quantitative resistance has also been shown to increase in transgenic plants carrying introduced R genes and matching avirulence genes, even though the latter do not express the hypersensitive cell death.

Race-Specific, Monogenic, R Gene, or Vertical Resistance

In many plant–pathogen combinations, especially those involving biotrophic oomycetes (downy mildews), fungi (powdery mildews, rusts), and many other fungi, e.g., *Cochliobolus*, *Magnaporthe*, *Cladosporium*, many bacteria, nematodes, and viruses, defense (resistance) of a host plant against many of its pathogens is through the presence of matching pairs of juxtaposed genes for disease in the host plant and the pathogen. The host plant carries one or few resistance genes (R) per pathogen capable of attacking it, while each pathogen carries matching genes for avirulence (A) for each of the R genes of the host plant. As explained in some detail later, the avirulence gene of the pathogen serves to trigger the host R gene into action. This then sets in motion a series of defense reactions that neutralize and eliminate the specific pathogen that carries the corresponding (matching) gene for avirulence (A), while the attacked and a few surrounding cells die. This type of defense or resistance is known as race-specific, hypersensitive response (HR), major gene, R gene, or vertical resistance. However, some R genes, e.g., Xa21 of rice, do not induce a visible HR.

PREEXISTING STRUCTURAL AND CHEMICAL DEFENSES

Preexisting Defense Structures

The first line of defense of a plant against pathogens is its surface, which the pathogen must adhere to and penetrate if it is to cause infection. Some structural defenses are present in the plant even before the pathogen comes in contact with the plant. Such structures include the amount and quality of wax and cuticle that cover the epidermal cells, the structure of the epidermal cell walls, the size, location, and shapes of stomata and lenticels, and the presence of tissues made of thick-walled cells that hinder the advance of the pathogen on the plant.

Waxes on leaf and fruit surfaces form a water-repellent surface, thereby preventing the formation of a film of water on which pathogens might be deposited and germinate (fungi) or multiply (bacteria). A thick mat of hairs on a plant surface may also exert a similar water-repelling effect and may reduce infection.

A thick cuticle may increase resistance to infection in diseases in which the pathogen enters its host only through direct penetration. Cuticle thickness, however, is not always correlated with resistance, and many plant varieties with cuticles of considerable thickness are invaded easily by directly penetrating pathogens.

The thickness and toughness of the outer wall of epidermal cells are apparently important factors in the resistance of some plants to certain pathogens. Thick, tough walls of epidermal cells make direct penetration by fungal pathogens difficult or impossible. Plants with such walls are often resistant, although if the pathogen is introduced beyond the epidermis of the same plants by means of a wound, the inner tissues of the plant are invaded easily by the pathogen.

Many pathogenic fungi and bacteria enter plants only through stomata. Although the majority of pathogens can force their way through closed stomata, some, like the stem rust of wheat, can enter only when stomata are open. Thus, some wheat varieties, in which the stomata open late in the day, are resistant because the germ tubes of spores germinating in the night dew desiccate due to evaporation of the dew before the stomata begin to open. The structure of the stomata, e.g., a very narrow entrance and broad, elevated guard cells, may also confer resistance to some varieties against certain of their bacterial pathogens.

The cell walls of the tissues being invaded vary in thickness and toughness and may sometimes inhibit the advance of the pathogen. The presence, in particular, of bundles or extended areas of sclerenchyma cells, such as are found in the stems of many cereal crops, may stop the further spread of pathogens such as stem rust fungi. Also, the xylem, bundle sheath, and sclerenchyma cells of the leaf veins effectively block the spread of some fungal, bacterial, and nematode pathogens that cause various “angular” leaf spots because of their spread only into areas between, but not across, veins. Xylem vessels seem to be involved more directly in the resistance and susceptibility to vascular diseases. For example, xylem vessel diameter and the proportion of large

vessels were strongly correlated with the susceptibility of elm to Dutch elm disease caused by the fungus *Ophiostoma novo-ulni*.

Preexisting Chemical Defenses

Although structural characteristics may provide a plant with various degrees of defense against attacking pathogens, it is clear that the resistance of a plant against pathogen attacks depends not so much on its structural barriers as on the substances produced in its cells before or after infection. This is apparent from the fact that a particular pathogen will not infect certain plant varieties even though no structural barriers of any kind seem to be present or to form in these varieties. Similarly, in resistant varieties, the rate of disease development soon slows down, and finally, in the absence of structural defenses, the disease is completely checked. Moreover, many pathogens that enter nonhost plants naturally or that are introduced into nonhost plants artificially, fail to cause infection, although no apparent visible host structures inhibit them from doing so. These examples suggest that defense mechanisms of a chemical rather than a structural nature are responsible for the resistance to infection exhibited by plants against certain pathogens.

Inhibitors Released by the Plant in Its Environment

Plants exude a variety of substances through the surface of their aboveground parts as well as through the surface of their roots. Some of the compounds released by certain kinds of plants, however, seem to have an inhibitory action against certain pathogens. **Fungitoxic exudates** on the leaves of some plants, e.g., tomato and sugar beet, seem to be present in sufficient concentrations to inhibit the germination of spores of fungi *Botrytis* and *Cercospora*, respectively, that may be present in dew or rain droplets on these leaves. Similarly, in the case of onion smudge, caused by the fungus *Colletotrichum circinans*, resistant varieties generally have red scales and contain, in addition to the red pigments, the phenolic compounds protocatechuic acid and catechol. In the presence of water drops or soil moisture containing conidia of the onion smudge fungus on the surface of red onions, these two fungitoxic substances diffuse into the liquid, inhibit the germination of the conidia, and cause them to burst, thus protecting the plant from infection. Both fungitoxic exudates and inhibition of infection are missing in white-scaled, susceptible onion varieties (Fig. 6-2). It was noticed that applications of acibenzolar-*S*-methyl (ASM) on sunflower reduced infection by the rust fungus *Puccinia helianthi* through the reduction of spore germination and appressorium formation. It was subsequently shown that ASM accomplished this by increasing the production and secretion by the plant on the leaf surface of coumarins and other toxic phenolics that inhibit spore germination and appressorium formation on the leaf surfaces on which they are present.

FIGURE 6-2 Onion smudge, caused by the fungus *Colletotrichum circinans*, develops on white onions but not on colored ones, which, in addition to the red or yellow pigment, also contain the phenolics protocatechuic acid and catechol, both of which are toxic to the fungus. (Photograph courtesy of G. W. Simone.)

Inhibitors Present in Plant Cells before Infection

It is becoming increasingly apparent that some plants are resistant to diseases caused by certain pathogens because of one or more inhibitory antimicrobial compounds, known as phytoanticipins, which are present in the cell before infection. Several **phenolic compounds**, **tannins**, and some fatty acid-like compounds such as **dienes**, which are present in high concentrations in cells of young fruits, leaves, or seeds, have been proposed as responsible for the resistance of young tissues to pathogenic microorganisms such as *Botrytis*. For example, increased 9-hexadecanoic acid in cutin monomers in transgenic tomato plants led to resistance of such plants to powdery mildew because these cutin monomers inhibit the germination of powdery mildew spores. Many such compounds are potent inhibitors of many hydrolytic enzymes, including the pectolytic-macerating enzymes of plant pathogens. As the young tissues grow older, their inhibitor content and their resistance to infection decrease steadily. Strawberry leaves naturally contain **(+)-catechin**, which inhibits infection by *Alternaria alternata* by blocking the formation of infection hyphae from haustoria although it allows both spore germination and appressoria formation. Several other types of preformed compounds, such as the saponins (glycosylated steroidal or triterpenoid compounds) **tomatine** in tomato and **avenacin** in oats, not only have antifungal membranolytic activity, they actually exclude fungal pathogens that lack enzymes

(saponinases) that break down the saponin from infecting the host. In this way, the presence or absence of saponin in a host and of saponinase in a fungus determines the host range of the fungus.

In addition to the simple molecule antifungal compounds listed earlier, several preformed plant proteins have been reported to act as inhibitors of pathogen proteinases or of hydrolytic enzymes involved in host cell wall degradation, to inactivate foreign ribosomes, or to increase the permeability of the plasma membranes of fungi.

For example, in a number of plants there is a family of low molecular weight proteins called phytocystatins that inhibit cysteine proteinases carried in the digestive system of nematodes and are also secreted by some plant pathogenic fungi. Constitutively present or transgenically introduced phytocystatins in plants reduce the size of nematode females and the number of eggs produced by females, thereby providing effective or significant control of several plants to root knot, cyst, reniform, and lesion nematodes.

Another type of compounds, the lectins, which are proteins that bind specifically to certain sugars and occur in large concentrations in many types of seeds, cause lysis and growth inhibition of many fungi. However, plant surface cells also contain variable amounts of hydrolytic enzymes, some of which, such as glucanases and chitinases, may cause the breakdown of pathogen cell wall components, thereby contributing to resistance to infection. The importance of either of these types of inhibitors to disease resistance is not currently known, but some of these substances are known to increase rapidly upon infection and are considered to play an important role in the defense of plants to infection.

DEFENSE THROUGH LACK OF ESSENTIAL FACTORS

Lack of Recognition between Host and Pathogen

A plant species either is a host for a particular pathogen, e.g., wheat for the wheat stem rust fungus, or it is not a host for that pathogen, e.g., tomato for wheat stem rust fungus. How does a pathogen recognize that the plant with which it comes in contact is a host or nonhost? Plants of a species or variety may not become infected by a pathogen if their surface cells lack specific **recognition factors** (specific molecules or structures) that can be recognized by the pathogen. If the pathogen does not recognize the plant as one of its host plants, it may not become attached to the plant or may not produce infection substances, such as enzymes, or structures, such as appressoria, penetration pegs, and haustoria, necessary for the establishment of infection. It is not known what types of molecules or structures are involved in the recognition of plants and pathogens, but it is thought that they probably include various types of oligosaccharides and polysaccharides, and proteins or glycoproteins. Also, it is not known to what extent these recognition phenomena are responsible for the success or failure of initiation of infection in any particular host–pathogen combination.

Lack of Host Receptors and Sensitive Sites for Toxins

In host–pathogen combinations in which the pathogen (usually a fungus) produces a host-specific toxin, the toxin, which is responsible for the symptoms, is thought to attach to and react with specific receptors or sensitive sites in the cell. Only plants that have such sensitive receptors or sites become diseased. Plants of other varieties or species that lack such receptors or sites remain resistant to the toxin and develop no symptoms.

Lack of Essential Substances for the Pathogen

Species or varieties of plants that for some reason do not produce one of the substances essential for the survival of an obligate parasite, or for development of infection by any parasite, would be resistant to the pathogen that requires it. Thus, for *Rhizoctonia* to infect a plant it needs to obtain from the plant a substance necessary for formation of a hyphal cushion from which the fungus sends into the plant its penetration hyphae. In plants in which this substance is apparently lacking, cushions do not form, infection does not occur, and the plants are resistant. The fungus does not normally form hyphal cushions in pure cultures but forms them when extracts from a susceptible but not a resistant plant are added to the culture. Also, certain mutants of *Venturia inaequalis*, the cause of apple scab, which had lost the ability to synthesize a certain growth factor, also lost the ability to cause infection. When, however, the particular growth factor is sprayed on the apple leaves during inoculation with the mutant, the mutant not only survives but it also causes infection. The advance of the infection, though, continues only as long as the growth factor is supplied externally to the mutant. In some host–pathogen combinations, disease develops but the amount of disease may be reduced by the fact that certain host substances are present in lower concentrations. For example, bacterial soft rot of potatoes, caused

by *Erwinia carotovora* var. *atroseptica*, is less severe on potatoes with low-reducing sugar content than on potatoes high in reducing sugars.

INDUCED STRUCTURAL AND BIOCHEMICAL DEFENSES

Recognition of the Pathogen by the Host Plant

Early recognition of the pathogen by the plant is very important if the plant is to mobilize the available biochemical and structural defenses to protect itself from the pathogen. The plant apparently begins to receive signal molecules, i.e., molecules that indicate the presence of a pathogen, as soon as the pathogen establishes physical contact with the plant (Fig. 6-3).

Pathogen Elicitors

Various pathogens, especially fungi and bacteria, release a variety of substances in their immediate environment that act as nonspecific elicitors of pathogen recognition by the host. Such nonspecific elicitors include toxins, glycoproteins, carbohydrates, fatty acids, peptides, and extracellular microbial enzymes such as proteases and pectic enzymes. In various host–pathogen combinations, certain substances secreted by the pathogen, such as *avr* gene products, *hrp* gene products, and suppressor molecules, act as specific pathogen elicitors of recognition by the specific host plant. In many cases, in which host enzymes break down a portion of the polysaccharides making up the pathogen surface or pathogen enzymes break down a portion of the plant surface polysaccharides, the released oligomers or monomers of the polysaccharides act as recognition elicitors for the plant.

Host Plant Receptors

The location of host receptors that recognize pathogen elicitors is not generally known, but several of those studied appear to exist outside or on the cell membrane, whereas others apparently occur intracellularly. In the powdery mildew of cereals, a soluble carbohydrate that acts as an elicitor from the wheat powdery mildew fungus *Blumeria graminis* f. sp. *tritici* is recognized by a broad range of cereals (barley, oat, rye, rice, and maize) in which it induces the expression of all defense-related genes tested and also induced resistance to subsequent attacks with the fungus. The elicitor alone, in

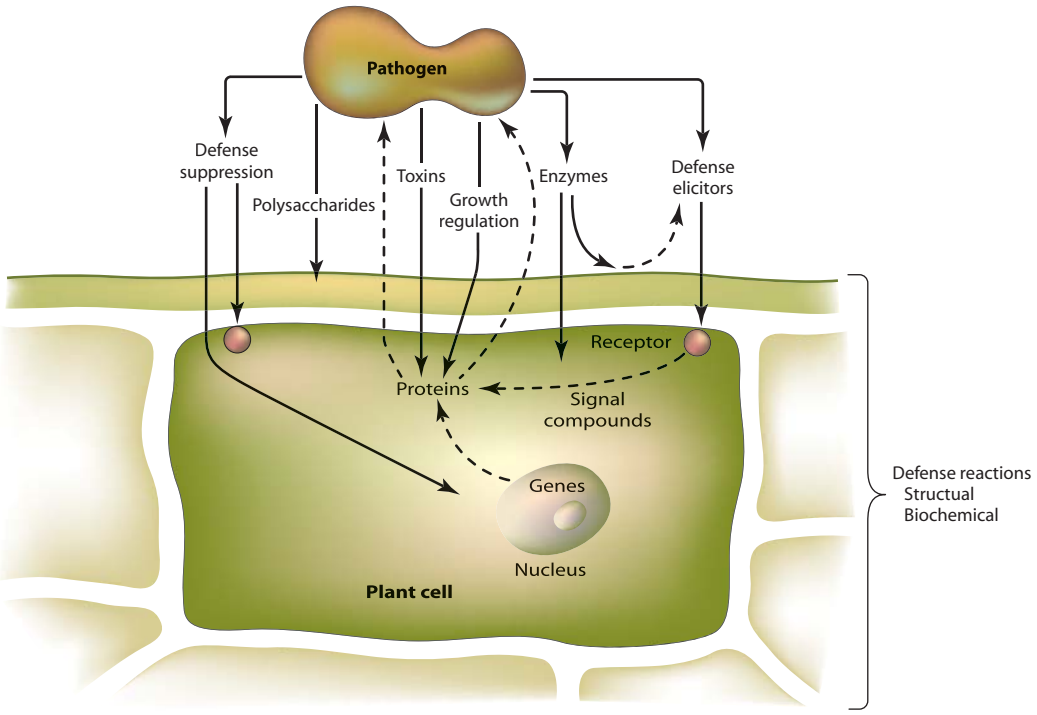


FIGURE 6-3 Schematic representation of pathogen interactions with host plant cells. Depending on its genetic makeup, the plant cell may react with numerous defenses, which may include cell wall structural defenses (waxes, cutin, suberin, lignin, phenolics, cellulose, callose, cell wall proteins) or biochemical wall, membrane, cytoplasm, and nucleus defense reactions. The latter may involve bursts of oxidative reactions, production of elicitors, hypersensitive cell death, ethylene, phytoalexins, pathogenesis-related proteins (hydrolytic enzymes, β-1,3-glucanases, chitinases), inhibitors (thionins, proteinase inhibitors, thaumatin-like proteins), and so on.

absence of the powdery mildew fungus, did not induce a hypersensitive response but it did induce an accumulation of thaumatin-like proteins in the various cereals.

Mobilization of Defenses

Once a particular plant molecule recognizes and reacts with a molecule (elicitor) derived from a pathogen, it is assumed that the plant "recognizes" the pathogen. Following such recognition, a series of biochemical reactions and structural changes are set in motion in the plant cell(s) in an effort to fend off the pathogen and its enzymes, toxins, etc. How quickly the plant recognizes the (presence of a) pathogen and how quickly it can send out its alarm message(s) and mobilize its defenses determine whether hardly any infection will take place at all (as in the hypersensitive response) or how much the pathogen will develop, i.e., how severe the symptoms (leaf spots, stem, fruit, or root lesions, etc.) will be, before the host defenses finally stop further development of the pathogen.

Transmission of the Alarm Signal to Host Defense Providers: Signal Transduction

Once the pathogen-derived elicitors are recognized by the host, a series of alarm signals are sent out to host cell proteins and to nuclear genes, causing them to become activated, to produce substances inhibitory to the pathogen, and to mobilize themselves or their products toward the point of cell attack by the pathogen. Some of the alarm substances and signal transductions are only intracellular, but in many cases the signal is also transmitted to several adjacent cells and, apparently, the alarm signal is often transmitted systemically to most or all of the plant.

The chemical nature of the transmitted signal molecules is not known with certainty in any host–pathogen combination. Several types of molecules have been implicated in intracellular signal transduction. The most common such signal transducers appear to be various protein kinases, calcium ions, phosphorylases and phospholipases, ATPases, hydrogen peroxide (H_2O_2), ethylene, and others. Systemic signal transduction, which leads to systemic acquired resistance, is thought to be carried out by salicylic acid, oligogalacturonides released from plant cell walls, jasmonic acid, systemin, fatty acids, ethylene, and others. Some natural or synthetic chemicals, such as salicylic acid and the synthetic dichloroisonicotinic acid, also activate the signaling pathway that leads to systemic acquired resistance against several diverse types of plant pathogenic viruses, bacteria, and fungi.

INDUCED STRUCTURAL DEFENSES

Despite the preformed superficial or internal defense structures of host plants, most pathogens manage to penetrate their hosts through wounds and natural openings and to produce various degrees of infection. Even after the pathogen has penetrated the preformed defense structures, however, plants usually respond by forming one or more types of structures that are more or less successful in defending the plant from further pathogen invasion. Some of the defense structures formed involve the cytoplasm of the cells under attack, and the process is called **cytoplasmic defense reaction;** others involve the walls of invaded cells and are called **cell wall defense structures**; and still others involve tissues ahead of the pathogen (deeper into the plant) and are called **histological defense structures**. Finally, the death of the invaded cell may protect the plant from further invasion. This is called the **necrotic** or **hypersensitive defense reaction** and is discussed here briefly, with more detailed treatment a little later.

Cytoplasmic Defense Reaction

In a few cases of slowly growing, weakly pathogenic fungi, such as weakly pathogenic *Armillaria* strains and the mycorrhizal fungi, that induce chronic diseases or nearly symbiotic conditions, the plant cell cytoplasm surrounds the clump of hyphae and the plant cell nucleus is stretched to the point where it breaks in two. In some cells, the cytoplasmic reaction is overcome and the protoplast disappears while fungal growth increases. In some of the invaded cells, however, the cytoplasm and nucleus enlarge. The cytoplasm becomes granular and dense, and various particles or structures appear in it. Finally, the mycelium of the pathogen disintegrates and the invasion stops.

Cell Wall Defense Structures

Cell wall defense structures involve morphological changes in the cell wall or changes derived from the cell wall of the cell being invaded by the pathogen. The effectiveness of these structures as defense mechanisms seems to be rather limited, however. Three main types of such structures have been observed in plant diseases. (1) The outer layer of the cell wall of parenchyma cells coming in contact with incompatible bacteria swells and produces an amorphous, fibrillar material that surrounds and traps the bacteria and prevents them from multiplying. (2) Cell walls thicken in response to several pathogens by producing what appears to be a cellulosic material. This material, however, is often infused with

phenolic substances that are cross-linked and further increase its resistance to penetration. (3) Callose **papillae** are deposited on the inner side of cell walls in response to invasion by fungal pathogens (see Figs. 2-8C and 2-8D). Papillae seem to be produced by cells within minutes after wounding and within 2 to 3 hours after inoculation with microorganisms. Although the main function of papillae seems to be repair of cellular damage, sometimes, especially if papillae are present before inoculation, they also seem to prevent the pathogen from subsequently penetrating the cell. In some cases, hyphal tips of fungi penetrating a cell wall and growing into the cell lumen are enveloped by cellulosic (callose) materials that later become infused with phenolic substances and form a sheath or lignituber around the hypha (Fig. 6-4).

Histological Defense Structures

Formation of Cork Layers

Infection by fungi or bacteria, and even by some viruses and nematodes, frequently induces plants to form several layers of cork cells beyond the point of infection (Figs. 6-5 and 6-6), apparently as a result of stimulation of the host cells by substances secreted by the pathogen. The cork layers inhibit further invasion by the pathogen beyond the initial lesion and also block the spread of any toxic substances that the pathogen may secrete. Furthermore, cork layers stop the flow of nutrients and water from the healthy to the infected area and deprive the pathogen of nourishment. The dead tissues, including the pathogen, are thus delimited by the cork layers

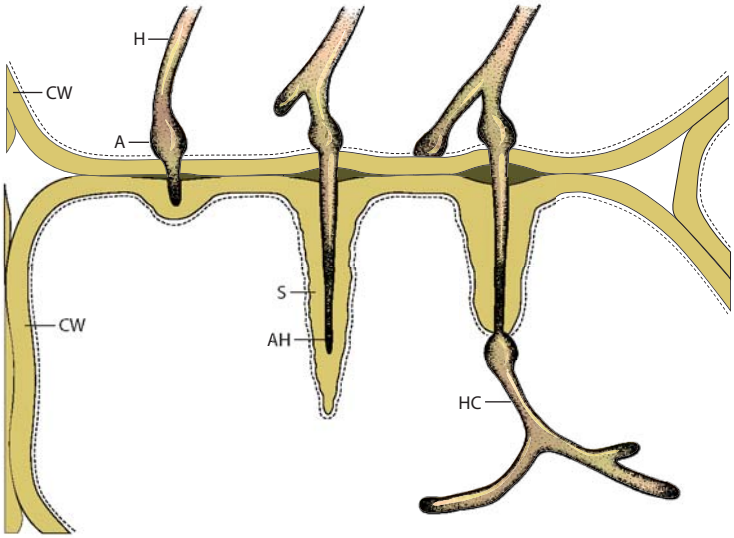


FIGURE 6-4 Formation of a sheath around a hypha (H) penetrating a cell wall (CW). A, appressorium; AH, advancing hypha still enclosed in sheath; HC, hypha in cytoplasm; S, sheath.

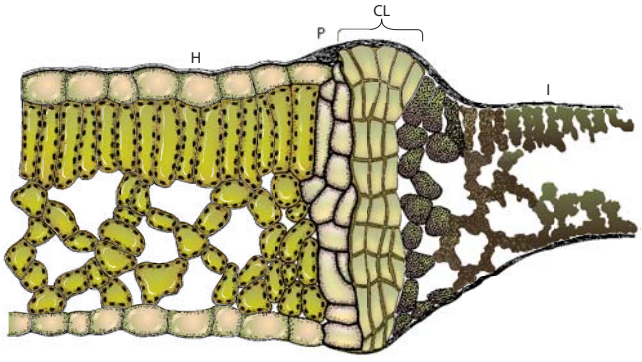


FIGURE 6-5 Formation of a cork layer (CL) between infected (I) and healthy (H) areas of leaf. P, phellogen. [After Cunningham (1928). *Phytopathology* **18**, 717–751.]

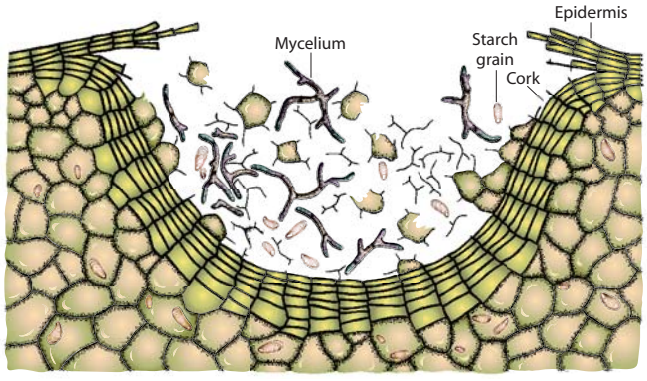
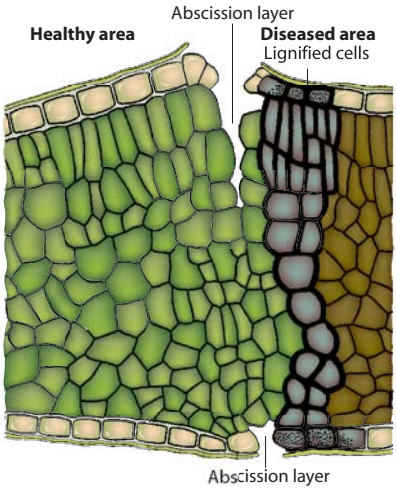


FIGURE 6-6 Formation of a cork layer on a potato tuber following infection with *Rhizoctonia*. [After Ramsey (1917). *J. Agric. Res.* **9**, 421–426.]

and may remain in place, forming necrotic lesions (spots) that are remarkably uniform in size and shape for a particular host–pathogen combination. In some host–pathogen combinations the necrotic tissues are pushed outward by the underlying healthy tissues and form scabs that may be sloughed off, thus removing the pathogen from the host completely. In tree cankers, such as those caused by the fungus *Seiridium cardinale* on cypress trees, resistant plant clones restrict growth of the fungus by forming ligno-suberized boundary zones, which included four to six layers of cells with suberized cell walls. In contrast, susceptible clones have only two to four layers of suberized cells and these are discontinuous, allowing repeated penetration by the fungus past the incomplete barrier.

Formation of Abscission Layers

Abscission layers are formed on young, active leaves of stone fruit trees after infection by any of several fungi, bacteria, or viruses. An abscission layer consists of a gap formed between two circular layers of leaf cells surrounding the locus of infection. Upon infection, the middle lamella between these two layers of cells is dissolved throughout the thickness of the leaf, completely cutting off the central area of the infection from the rest of the leaf (Fig. 6-7). Gradually, this area shrivels, dies, and sloughs off, carrying with it the pathogen. Thus, the plant, by discarding the infected area along with a few yet uninfected cells, protects the rest of the leaf tissue from being invaded by the



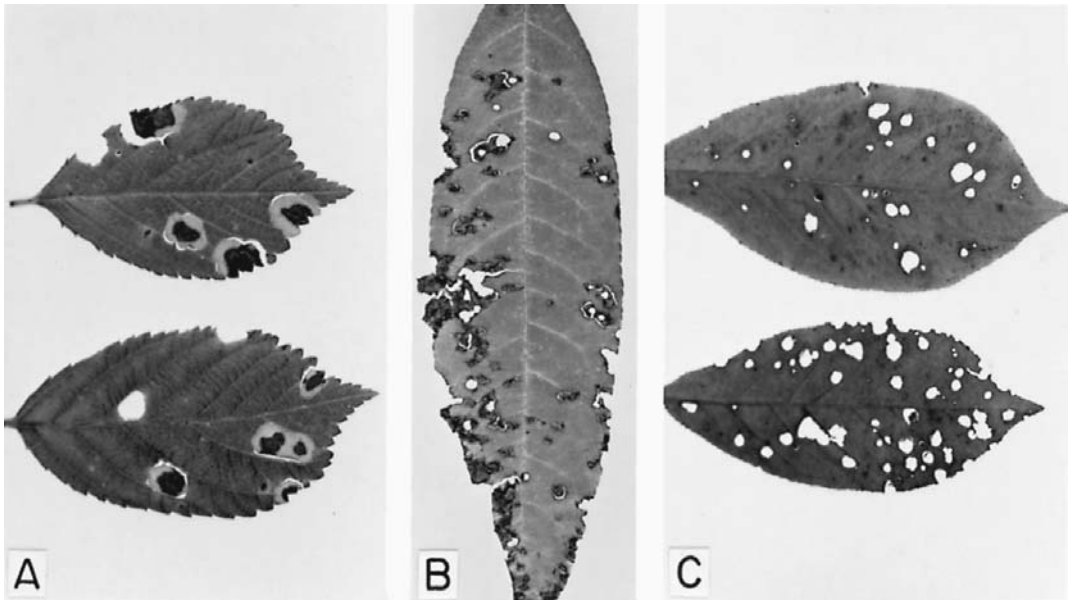


FIGURE 6-7 Schematic formation of an abscission layer around a diseased spot of a *Prunus* leaf. [After Samuel (1927).] (A–C) Leaf spots and shot holes caused by *Xanthomonas arboricola* pv. *pruni* bacteria on (A) ornamental cherry leaves; characteristic broad, light green halos form around the infected area before all affected tissue falls off, (B) on peach, and (C) on plum. The shot hole effect is particularly obvious on the plum leaves.

pathogen and from becoming affected by the toxic secretions of the pathogen.

Formation of Tyloses

Tyloses form in xylem vessels of most plants under various conditions of stress and during invasion by most of the xylem-invading pathogens. Tyloses are overgrowths of the protoplast of adjacent living parenchymatous cells, which protrude into xylem vessels through pits (Fig. 6-8). Tyloses have cellulosic walls and may, by their size and numbers, clog the vessel completely. In some varieties of plants, tyloses form abundantly and quickly ahead of the pathogen, while the pathogen is still in the young roots, and block further advance of the pathogen. The plants of these varieties remain free of and therefore resistant to this pathogen. Varieties in which few, if any, tyloses form ahead of the pathogen are susceptible to disease.

Deposition of Gums

Various types of gums are produced by many plants around lesions after infection by pathogens or injury. Gum secretion is most common in stone fruit trees but occurs in most plants. The defensive role of gums stems from the fact that they are deposited quickly in the intercellular spaces and within the cells surrounding the locus of infection, thus forming an impenetrable barrier that completely encloses the pathogen. The pathogen then becomes isolated, starves, and sooner or later dies.

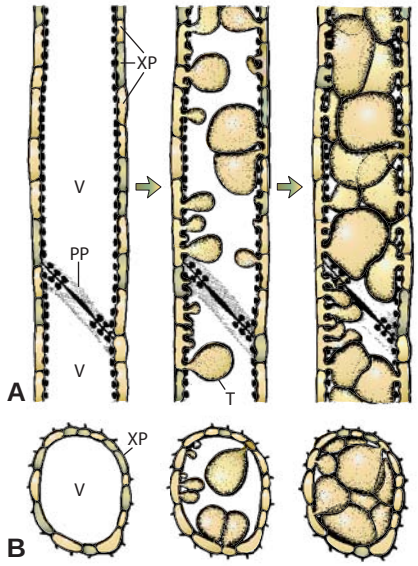


FIGURE 6-8 Development of tyloses in xylem vessels. Longitudinal (A) and cross section (B) views of healthy vessels (left) and of vessels with tyloses. Vessels at right are completely clogged with tyloses. PP, perforation plate; V, xylem vessel; XP, xylem parenchyma cell; T, tylosis.

Necrotic Structural Defense Reaction: Defense through the Hypersensitive Response

The hypersensitive response is considered a biochemical rather than a structural defense mechanism but is described here briefly because some of the cellular responses that accompany it can be seen with the naked eye or with the microscope. In many host–pathogen combinations, as soon as the pathogen establishes contact with the cell, the nucleus moves toward the invading pathogen and soon disintegrates. At the same time, brown, resin-like granules form in the cytoplasm, first around the point of penetration of the pathogen and then throughout the cytoplasm. As the browning discoloration of the plant cell cytoplasm continues and death sets in, the invading hypha begins to degenerate (Fig. 6-9). In most cases the hypha does not grow out of such cells, and further invasion is stopped. In bacterial infections of leaves, the hypersensitive response results in the destruction of all cellular membranes of cells in contact with bacteria, which is followed by desiccation and necrosis of the leaf tissues invaded by the bacteria.

Although it is not quite clear whether the HR is the cause or the consequence of resistance, this type of necrotic defense is quite common, particularly in diseases caused by obligate fungal parasites and by viruses (Fig. 6-10A), bacteria (Fig. 6-10B), and nematodes. Apparently, the necrotic tissue not only isolates the parasite from the living substance on which it depends for its nutrition and, thereby, results in its starvation and death, but, more importantly, it signifies the concentration of numerous biochemical cell responses and antimicrobial substances that neutralize the pathogen. The faster the host cell dies after invasion, the more resistant to infection the plant seems to be. Moreover, through the signaling compounds and pathways developed during the hypersensitive response, the latter serves as the springboard for localized and systemic acquired resistance.

INDUCED BIOCHEMICAL DEFENSES

Induced Biochemical Nonhost Resistance

As mentioned earlier, nonhost resistance is the resistance that keeps a plant protected from pathogens that are, through evolution, incompatible with that host. Although the nature of nonhost resistance is unknown, for a pathogen it can be as big a gap to bridge as the difference between the features of a potato plant and an oak tree, or as close as the difference between the features of potato and tomato, or barley and wheat. It appears, however, that in some plant/pathogen

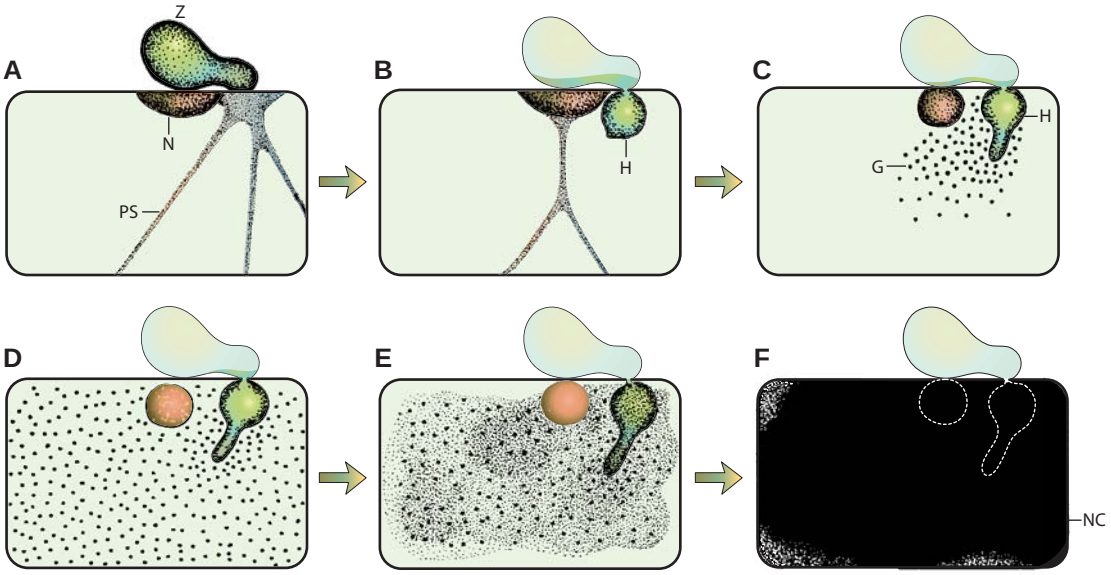


FIGURE 6-9 Stages in the development of the necrotic defense reaction in a cell of a very resistant potato variety infected by *Phytophthora infestans*. N, nucleus; PS, protoplasmic strands; Z, zoospore; H, hypha; G, granular material; NC, necrotic cell. [After Tomiyama (1956). *Ann. Phytopathol. Soc. Jpn.* **21**, 54–62.]

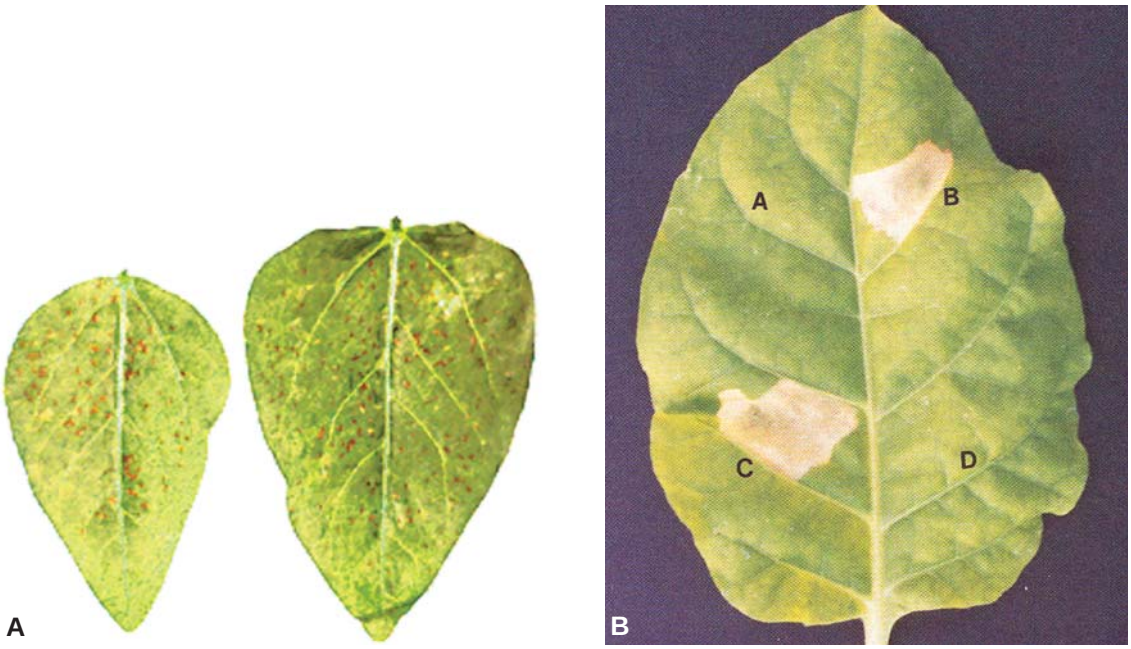


FIGURE 6-10 (A) Hypersensitive response (HR) expressed on leaves of a resistant cowpea variety following sap inoculation with a strain of a virus that causes local lesions (in this case, *alfalfa mosaic virus*). The virus remains localized in the lesions. (B) Tobacco leaf showing typical hypersensitive responses (white areas) 24 hours after injection with water (A) or with preparations of bacterial strains B, C, and D. Strain (B), which does not infect tobacco, and (C), which carries a *hrp* (hypersensitive response and pathogenicity) gene, both induced the hypersensitive response, whereas the third strain (D), a mutant of C that lacked the *hrp* gene, did not. [From Mukherjee *et al.* (1997). *Mol. Plant-Microbe Interact.* **10**, 462–471.]

interactions of taxonomically unrelated plants (e.g., potato and oak or oak and wheat), nonhost resistance is controlled by constitutive defenses and/or defenses induced by nonspecific stimuli in a nonspecific manner. Such defenses include physical topography and the structures present on the plant, the presence of toxic or the absence of essential compounds, and so on. In other plant/pathogen combinations, in which the plants are taxonomically related (e.g., potato and tomato, barley and wheat), nonhost resistance involves primarily inducible defenses elicited by the recognition of pathogen-specific molecules. Some cases of nonhost resistance, however, seem to be controlled by a single gene.

Some examples of questionable nonhost resistance include the resistance of the nonhost pea to the *Pseudomonas syringae* pv. *syringae* bacterium, which infects bean but not pea. The reaction occurs when that bacterium carries a gene that is responsible for elicitation of a potentially defensive response in the normally nonhost pea, that is expressed as a visible hypersensitive response. In another example, the potato late blight fungus *Phytophthora infestans*, normally does not infect the tobacco species *Nicotiana benthamiana*. The nonhost resistance of the tobacco species, however, is lost if the pathogen does not carry an "avirulence-like gene," which produces a protein that elicits cell death in the tobacco. This is unique in that in other plant/pathogen combinations, the absence of a single "nonhost avirulence gene" does not make the nonhost plant susceptible. It would appear, therefore, that if the cell death response to the elicitor controlled by the avirulence gene really contributes to resistance, then the nonhost resistance in such situations is controlled by more than one component. In still another case, nonhost resistance in some cereals [wheat to powdery mildew strains from another cereal (barley), or in barley to *Puccinia* rust races from wheat], involves similar gene-for-gene interactions and nonhost resistance occurs through defense mechanisms involving recognition of an elicitor and development of a hypersensitive response. Disease resistance does not always involve pathogen recognition events, but, especially in polygenic or quantitative resistance, it may involve directly various structural or chemical defense mechanisms. This also happens in some cases of nonhost resistance, e.g., in oat roots to the wheat fungus *Gaeumannomyces graminis* f. sp. *tritici*, while they are susceptible to the oat fungus *G. graminis* f. sp. *avenae*. The nonhost resistance of oat roots to the wheat fungus is caused by the presence of the saponin compound avenacin in the oat roots, which is toxic to the fungus. This compound is also toxic to the oat fungus, but the latter produces an enzyme that detoxifies the saponin in oat roots and can infect them. The nonhost resistance to the wheat fungus, however, is compromised in saponin-deficient mutants in which the wheat fungus causes a successful infection. This shows that nonhost resistance in some plant/microbe interactions is caused by a direct defense mechanism rather than by recognition events.

In all these examples, the pathogen or the host is already closely related and nearly fully adopted to the characteristics of nonhost resistance presented to it. In less related plants or pathogens, however, in which true nonhost resistance is found routinely, it is more likely to be the result of effective nonspecific defenses such as physical characteristics and nonspecific responses to wounding and damage done by the pathogen during attempted invasion than to defenses elicited by specific recognition events. There is also, however, the case of pathogens that have alternate hosts, such as wheat stem rust and barberry and cedar apple rust on apple and cedar. These are, perhaps, interesting from an evolutionary point of view because, presumably, before the second of the alternate hosts that became a host, it was surely a nonhost. How the rust fungus bridged the two taxonomically extremely different hosts is not known. The change in ploidy (from haploid to diploid and back to haploid) was probably involved, but how the fungus broke the nonhost resistance of the other host and how it used the nonresistant host as a completely cooperative host is still a mystery.

The present consensus is that plants that exhibit nonhost resistance against pathogens of other plants do not need to carry resistance genes that recognize these pathogens because they carry genes that provide the plants with nonspecific defenses that are fully effective in protecting the plant from these pathogens. However, it may be possible that nonhost resistance, along with polygenic and monogenic host resistance, forms a continuum of resistance that begins to overlap as the taxonomic (evolutionary) distance between host and nonhost plants becomes closer and results in a complex and continuous network of plant/pathogen interactions.

Induced Biochemical Defenses in Quantitative (Partial, Polygenic, General, or Horizontal) Resistance

In quantitative (partial, polygenic, multigenic, general, field, durable, or horizontal) resistance, plants depend on the action of numerous genes, expressed constitutively or upon attack by a pathogen (induced resistance). These genes provide the plants with defensive structures or toxic substances that slow down or stop the advance of the pathogen into the host tissues and reduce the damage caused by the pathogen. Quantitative resistance is particularly common in diseases caused

by nonbiotrophic pathogens. Quantitative resistance may vary considerably, in some cases being specific against some of the strains of a pathogen, in others being effective against all strains of a pathogen, or providing resistance against more than one pathogen. Genes for quantitative resistance are present and provide a basal level of resistance to all plants against all pathogens regardless of whether the plant also carries major (or R) genes against a particular pathogen.

Function of Gene Products in Quantitative Resistance

Unlike most major (or R) genes involved in monogenic resistance, which appear to code for components that help the host recognize the pathogen and to subsequently express the hypersensitive response, genes for quantitative resistance seem to be involved directly in the expression or production of some sort of structural or biochemical defense. Quantitative resistance defenses are basically the same ones that follow the hypersensitive response in monogenic resistance; in quantitative resistance, however, defenses generally do not follow a hypersensitive response and cell death because the latter do not usually occur in quantitative resistance. Genes involved in quantitative resistance are present in the same areas of plant chromosomes that contain the genes involved in defense responses, such as the production of phenylalanine ammonia lyase, hydroxyproline-rich glycoproteins, and pathogenesis–related proteins. The defenses in quantitative resistance, however, develop slower and perhaps reach a lower level than those in the race-specific (R gene) resistance. Quantitative resistance is also affected much more by changes in the environment, mostly of changes in temperature during the various stages of development of resistance.

Mechanisms of Quantitative Resistance

Studies of defense mechanisms in diseases with quantitative resistance are few and far between. For example, in the early blight of tomato caused by the fungus *Alternaria solani*, all resistant tomato lines had higher constitutive levels of the pathogenesis-related proteins chitinase and β-1,3-glucanase than the susceptible lines. Also, preparations of constitutive enzymes from quantitatively resistant, but not from susceptible, tomato plants could release elicitors of plant cell death, and possibly of a hypersensitive response, from the cell walls of the fungus. These results show that, in this host–plant interaction, the defense responses involve the production of higher levels of pathogenesis-related proteins in resistant plants, and the same plants may also induce the pathogen to produce elicitor molecules that potentiate a more aggressive defense response through the induction of cell death and a hypersensitive-like response. The latter defenses are produced in a manner not unlike that in a specific host–pathogen interaction, but in the absence of host R genes. In the quantitatively controlled resistance of the soybean–*Phytophthora* interaction, soybean tissues actually caused the release of phytoalexin elicitors from the cell walls of the fungus, again showing that the plant can play an important role in forcing the release of defense-triggering signals from the pathogen. Finally, when five cabbage varieties of different resistance levels were inoculated with a strain of the cabbage black rot bacterium *Xanthomonas campestris* pv. *campestris*, two varieties were resistant, one was partially resistant, and two were susceptible. In all varieties there was an increase in the total oxidant activity of peroxidase and superoxide dismutase, accumulation of peroxidases, and lignin deposition. The increases, however, were greater and generally occurred earlier in resistant than in susceptible varieties. However, activity of the antioxidant catalase decreased in both resistant and susceptible varieties, but it decreased more in the resistant variety. The resistant varieties also produced new isozymes of peroxidase and superoxide dismutase that were not produced by the susceptible variety. These results suggest that in the cabbage–*X. campestris* pv. *campestris* system there is a multilevel resistance similar to a hypersensitive response, although the onset of this response was delayed when compared to the classical HR. In barley leaves infected with the fungus *Drechslera teres*, as many as eight pathogenicity-related proteins with thaumatin-like activity were detected.

Effect of Temperature on Quantitative Resistance

Quantitative resistance is often affected greatly by the temperature in the environment. This effect, however, is not unique to plants with quantitative resistance, as even in plants with monogenic (R) gene resistance, the resistance of the host may be changed drastically by changes in temperature. For example, in R resistance-carrying wheat, a change in temperature from 18 to 30°C changes the reaction of wheat plants carrying the Sr6 R gene from rust resistant to rust susceptible. Also, resistance to rust and powdery mildew was increased in pea and barley, respectively, by low-temperature hardening of these grain crops. However, a brief "heat shock" may cause a brief period of susceptibility of wheat plants to rust, while it induces resistance to powdery mildew in barley and to cucumber scab, caused by the fungus *Cladosporium cucumerinum*, in cucumber, in which it also causes an increase in peroxidase activity. There are numerous reports of different plants synthesizing a

variety of pathogenesis-related (PR) proteins in response to abiotic (low temperature, drought, pollution, wounding) as well as to biotic (fungi, bacteria, etc.) stresses. Some of the PR proteins include PR-1, PR-2 (β-1,3-glucanases), PR-3 (chitinases), and PR-5 (thaumatin-like proteins), as well as peroxidases. Stressed plants also increase the production of phenylalanine ammonia lyase (PAL), which is involved in the production of phytoalexins.

In a detailed study of the effect of cold hardening of wheat on its quantitative resistance to infection by the snow mold fungi, it was found that cold hardening increases the resistance of wheat to snow mold and also induces changes in the expression (activity) of genes associated with PR proteins and other defense responses, some of them associated with induced systemic resistance. The most abundant PR proteins produced were chitinase, followed by PAL, β-1,3-glucanase, PR-1, and peroxidase. Similar PR proteins were produced by plants receiving cold treatment only, but the level of these proteins was lower and appeared later than when the plants were also infected by the snow mold fungi. It is apparent, therefore, that this biotic stress induces resistance and that the resistance is further augmented by the fungal infection. This type of resistance has characteristics similar to those of pathogen- and salicylic acid-induced resistance, including the expression of PR genes and further enhancement of defense-associated genes following the infection by a pathogen.

It should be noted in the aforementioned paragraphs that all plants produce PR and other defense-associated proteins constitutively and/or following induction by biotic and abiotic agents. In some host/pathogen combinations the level of constitutively produced PR proteins can be correlated with the level of partial resistance of the cultivars to the pathogen. There is no proof, however, that this correlation is meaningful, especially since some varieties lack the constitutive production of certain PR proteins and yet the plants exhibit partial resistance. It is possible, of course, that plants in the latter varieties have a means of upregulating PR gene expression upon infection that the other varieties lack. As was mentioned already, quantitative resistance depends (a) on the preexisting and induced structural and biochemical defenses provided by dozens and, probably, hundreds of defense-associated genes, (b) on PR proteins, which may provide another significant portion of the overall defenses, and (c) on the possible ability of PR proteins to potentiate a more aggressive response by plant cells to the pathogen invasion by inducing the pathogen to release molecules eliciting host defenses in the absence of a gene-for-gene relationship between host and pathogen.

INDUCED BIOCHEMICAL DEFENSES IN THE HYPERSENSITIVE RESPONSE (RACE-SPECIFIC, MONOGENIC, R GENE, OR VERTICAL) RESISTANCE

The Hypersensitive Response

The hypersensitive response, often referred to as HR, is a localized induced cell defense in the host plant at the site of infection by a pathogen (Fig. 6-10A). HR is the result of quick mobilization of a cascade of defense responses by the affected and surrounding cells and the subsequent release of toxic compounds that often kill both the invaded and surrounding cells and, also, the pathogen. The hypersensitive response is often thought to be responsible for limiting the growth of the pathogen and, in that way, is capable of providing resistance to the host plant against the pathogen. An effective hypersensitive response may not always be visible when a plant remains resistant to attack by a pathogen, as it is possible for the hypersensitive response to involve only single cells or very few cells and thereby remain unnoticed. Under artificial conditions, however, injection of several genera of plant pathogenic bacteria into leaf tissues of nonhost plants results in the development of a hypersensitive response. The artificially induced HR consists of large leaf sectors becoming water soaked at first and, subsequently, necrotic and collapsed within 8 to 12 hours after inoculation (Fig. 6-10B). The bacteria injected in the tissues are trapped in the necrotic lesions and generally are killed rapidly. The HR may occur whenever virulent strains of plant pathogenic bacteria are injected into nonhost plants or into resistant varieties and when avirulent strains are injected into susceptible cultivars. Although not all cases of resistance are due to the hypersensitive response, HR-induced resistance has been described in numerous diseases involving obligate parasites (fungi, viruses, mollicutes, and nematodes), as well as nonobligate parasites (fungi and bacteria).

The hypersensitive response is the culmination of the plant defense responses initiated by the recognition by the plant of specific pathogen-produced signal molecules, known as elicitors. Recognition of the elicitors by the host plant activates a cascade of biochemical reactions in the attacked and surrounding plant cells and leads to new or altered cell functions and to new or greatly activated defense-related compounds (Fig. 6-11). The most common new cell functions and compounds include a rapid burst of reactive oxygen species, leading to a dramatic increase of oxidative reactions; increased ion movement, especially of K^+ and H^+ through the cell membrane; disruption of membranes and loss of

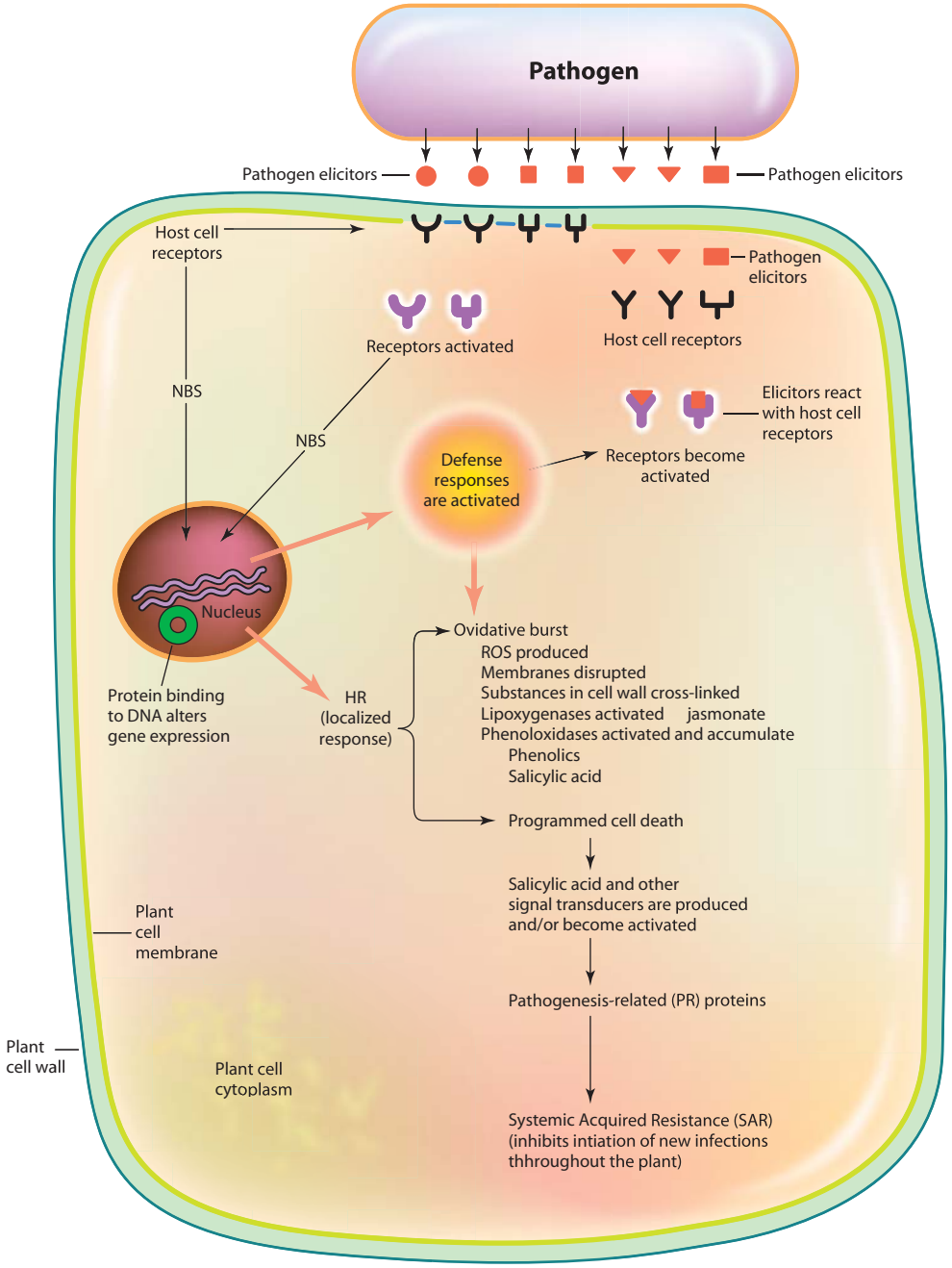


FIGURE 6-11 Diagram of the hypothetical steps in the hypersensitive response defense of plants following interaction of an elicitor molecule produced by a pathogen avirulence gene with a receptor molecule produced by the matching host R gene.

cellular compartmentalization (Fig. 6-12); cross-linking of phenolics with cell wall components and strengthening of the plant cell wall; transient activation of protein kinases (wounding-induced and salicylic acid-induced kinases); production of antimicrobial substances such as phenolics (phytoalexins); and formation of antimicrobial so-called pathogenesis-related proteins such as chitinases.

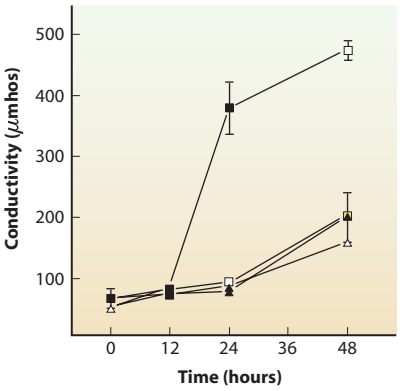


FIGURE 6-12 Disruption of cell membranes leads to a dramatic increase in cell electrolyte leakage, measured by increased current conductivity. This occurs when a resistant variety (■) containing an R gene is inoculated with pathogens containing an avirulence gene corresponding to the R gene. Same variety inoculated with a pathogen lacking the avirulence gene (□); another variety, susceptible to both pathogens (△, ▲). [From Whalen *et al.* (1993). *Mol. Plant-Microbe Interact.* **6**, 616–627.]

The hypersensitive response occurs only in specific host–pathogen combinations in which the host and the pathogen are incompatible, i.e., the pathogen fails to infect the host. It is thought that this happens because of the presence in the plant of a resistance gene (R), which recognizes and is triggered into action by the elicitor molecule released by the pathogen. The pathogen-produced elicitor is, presumably, the product of a pathogen gene, which, because it triggers the development of resistance in the host that makes this pathogen avirulent, is called an avirulence gene. For several pathogens, primarily bacteria, avirulence genes have been isolated and the proteins coded by them have been identified. The first avirulence gene product to be identified was the protein of the avirulence gene D (*avr*D) of the bacterium *Pseudomonas syringae* pv. *glycinea*. This was shown to be an enzyme involved in the synthesis of substances known as syringolides. The latter have the ability to elicit the hypersensitive response in soybean varieties that carry the resistance gene D complementary to *avr*D of the bacterium.

More than 20 resistance (R) genes have been isolated from a variety of plants such as corn, tomato, tobacco, rice, flax, and *Arabidopsis*, a model plant used for experimental purposes. The corn R gene Hm1 for northern leaf spot codes for an enzyme that inactivates the HC toxin of the fungus *Cochliobolus carbonum*, the cause of northern leaf spot of corn, whereas the tomato gene Pto, that confers resistance to the tomato speck-causing bacterium *Pseudomonas syringae* pv. *tomato*, codes for a protein kinase enzyme that most likely plays a role in signal transduction by triggering other enzymes into action. The functions of the proteins encoded by most other R genes are not known with certainty, but most of them contain domains, such as leucine-rich repeats, found in proteins involved in protein–protein interactions. Proteins coded by the tobacco R gene, which protects against tobacco mosaic virus, and the *Arabidopsis* R gene, which protects against a leaf-spotting bacterium, appear to be present in the plant cell cytoplasm and, therefore, probably recognize pathogen elicitors that reach the cytoplasm. However, the protein encoded by the tomato R gene *Cf*-9, which provides resistance against race 9 of the leaf mold fungus *Cladosporium fulvum*, and the rice R gene XA21, which provides resistance against many races of the leaf-spotting bacterium *Xanthomonas oryzae*, are transmembrane receptor-like proteins with a short anchor and a protein kinase, respectively. The last two R gene products, therefore, apparently recognize pathogen-produced molecules as they approach or come in contact with the plant cell membrane.

Genes Induced during Early Infection

Through recent methodology [suppression subtractive hybridization (SSH), cDNA library construction, expressed sequence tag (EST) determination, large-scale DNA sequencing, and DNA microarrays], it is now possible to detect and identify numerous plant genes (or ESTs) and their organization, including those induced during compatible or incompatible interactions between plant pathogens and their hosts. DNA microarrays, especially, can provide extremely useful information on the expression patterns of thousands of genes in parallel. Earlier studies, for example, of a compatible interaction of *Phytophthora infestans* and potato, 43 genes appeared to be induced, 10 of which showed increased activity as a result of the infection. Some of them were homologous to genes already known to be activated during infection, e.g., for β-1,3-glucanase, some have homology to enzymes involved in detoxification, and some code for proteins that had not been reported earlier to be induced by infection. When genes expressed by rice seedlings 48 hours after inoculation with the fungus *Magnaporthe grisea* were examined, of 619 randomly selected clones, 359 expressed sequence tags that had not been described before. When 124 of 260 ESTs that showed moderate and high similarity were organized according to their suspected function, the largest group (21%) contained (24) stress or defense response genes. When looked at from a different angle, many of the genes were new and not described previously, but several had been described before and were known to be involved in the infection process; one, for example, being the rice peroxidase gene, which is expressed during the infection of rice with the bacterial blight pathogen *Xanthomonas oryzae* pv. *oryzae*.

In more recent studies, almost 2,400 genes of Arabidopsis were examined for transcriptional changes that may occur after inoculation with the incompatible fungal pathogen *Alternaria brassicicola* or after treatment with defense signaling compounds such as salicylic acid (SA), methyl jasmonate (MJ), or ethylene. More than 700 of the genes exhibited transcriptional changes in response to one or more of the treatments. Based on similarity of the sequences of these genes to known gene sequences, the majority of the activated genes were already known, but an additional 106 genes were also activated. Treatments with salicylic acid and methyl jasmonate activated 192 and 221 genes, respectively, but they also repressed the transcription of 131 and 96 genes, respectively. Of the identified genes that were activated, a number of them are involved in the oxidative burst, in antimicrobial defense, cell wall modification, phytoalexin production, and defense signal transduction. There appears to be a high level of interaction among signaling pathways regulated by pathogen infection or by treatment with SA, MJ, or ethylene. For example, of 2,375 ESTs analyzed simultaneously, 169 were regulated by more than one pathway. Of these, 55 genes were coinduced and 28 genes were corepressed by SA and MJ in local tissue, but only 6 genes were coinduced in both local and systemic tissue.

Functional Analysis of Plant Defense Genes

Expression of dozens or hundreds of genes at a particular physiological state, such as at a certain time interval after inoculation with a pathogen or a related treatment, implies the involvement of these genes in that physiological state. Determination, however, of which specific gene is responsible for a certain function requires that the study of the function of each gene be carried out individually. This is a very difficult task, partly because of the large number of genes contributing to the same function and because many of the same functions are carried out by several different genes. Also, several plant gene families consist of 100 or more members, and in some gene families related to transcription factors, most of the genes are particularly associated with defense responses. Nevertheless, candidate genes identified in microarray experiments can be subjected to detailed functional analysis *in planta* through several strategies, including posttranscriptional silencing, overexpression of genes, gene knockout experiments using insertional mutagenesis via transposon or T-DNA, through promoter trap strategies, and others.

The generation of transgenic plants for the functional analysis of genes is both time-consuming and may show high variation of transgene expression. The identification of transcription factors and their binding sites in the promoter regions of defense-related genes is also critical for understanding how defense gene expression is regulated. It is now possible to identify novel regulatory elements in the promoter regions of coregulated genes with bioinformatics tools. Genes that participate in the same biochemical, cellular, or developmental processes may be controlled by the same sets of transcription factors and, therefore, promoter sequences of such genes may also have some common regulatory sequences.

Classes of R Gene Proteins

The various plant R genes, regardless of the type of pathogen (bacterial, fungal, or viral) to which they confer resistance, have many structural similarities. It appears that most, if not all, R genes exist as clustered gene families. So far, depending on structure and function, R genes can be subdivided into five classes (Fig. 4-14, Table 4-5) (The R-like gene *Hm1*, which encodes a detoxifying enzyme, does not fit and does not follow the gene-for-gene concept.) (1) R genes, like *Pto*, encode a serine–threonine protein kinase that plays a role in signal transduction. (2) R genes, like *Xa21* of rice, which encode a transmembrane protein rich in extracellular leucine repeats and a cytoplasmic serine–threonine kinase, function as receptors of kinase-like proteins and transmit the signal to phosphokinases for further amplification. (3) R genes, like the tobacco N^1 gene, the flax L^6 gene, and the RPP5 *Arabidopsis* gene, encode proteins that are cytoplasmic. These cytoplasmic proteins, in addition to leucine-rich repeats, also have a site that binds to nucleotides (NBS) and a domain (TIR) with significant homology to the Toll/interleukin 1 receptor; such proteins may serve as receptors that activate the translocation of a transcription factor from the cytoplasm to the nucleus where it activates transcription of the genes related to hypersensitive response. (4) Another group of cytoplasmic R proteins also have LRR and NBS, but have a coiled coil domain that contains a putative leucine zipper domain, such as in RPS2 and RPM1. (5) R genes, like the tomato *Cf2–Cf9* genes, encode proteins that consist primarily of leucine-rich repeats and are located outside the cell membrane but are attached to the membrane with a transmembrane anchor. Such R gene-coded proteins may serve as receptors for the extracellular or intracellular elicitor molecules produced as the result of expression of the corresponding *avr* gene. For example, in the case of *avr9*, the elicitor molecule is a peptide consisting of 22 amino acids and binds to the receptor product of the *Cf9* R gene. A potential sixth class of R proteins may be coded by Arabidopsis genes RPW8.1 and RPW8.2, which individually provide resistance against a broad

range of powdery mildew pathogens. RPW8 proteins have limited homology to NBS-LRR proteins, but induce localized, salicylic acid-dependent defenses similar to those induced by R genes that control specific resistance, with the important difference that RPW8 genes induce broad resistance.

Depending on their structural characteristics, plant receptors can be classified under different categories, such as receptor-like protein kinases (RLKs), histidine kinase receptors, and receptors with different numbers of transmembrane domains. The most important receptors in relation to their recognition of a pathogen are RLKs, of which, apparently, there are hundreds in each plant species. RLKs have an extracellular domain that seems to be involved in signal recognition, a transmembrane domain, and a cytoplasmic kinase domain, which may be the one that initiates a cascade of signal transduction in the cell. All the RLKs studied so far are of the serine–threonine type and, depending on the structural characteristics of the extracellular domain, the receptor-like protein kinases have been subdivided into different categories (Fig. 6-13). The variety of RLKs and the large number of them present in plants suggest that RLKs may be involved in the recognition of many and variable stimuli, in addition to those in plant–pathogen interactions. For example, some RLKs are the products of R genes, e.g., Xa21 from rice that confers resistance to the bacterium *Xanthomonas oryzae* pv. *oryzae*; several R genes actually encode cytoplasmic proteins that are related to RLKs, such as the kinase encoded by the Pto gene, which is involved in resistance against *P. syringae*. Several RLKs are involved in the plant defense responses to pathogen attacks. Some RLKs are induced by oxidative stress, salicylic acid, and pathogen attack, wounding, and bacterial infection. Furthermore, there are RLKs that structurally resemble pathogenesis-related (PR) proteins, chitinase, or have lectin-like motifs. By far the best-studied receptor system for a general pathogen elicitor is the flagellin receptor, which seems to be very similar in both plant and animal systems.

Recognition of Avr Proteins of Pathogens by the Host Plant

Although the number of R genes for which the matching *Avr* gene has been cloned is increasing steadily, in very few of the studied host–pathogen interactions has it been shown that there is a direct interaction of R and *Avr* gene products. In many host–pathogen relationships there is no physical interaction between R and Avr proteins and it appears that the recognition of Avr proteins

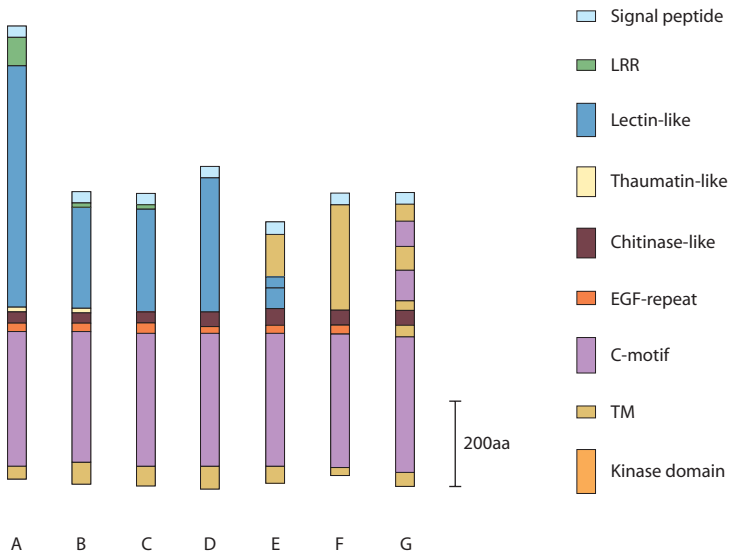


FIGURE 6-13 Schematic diagram of plant receptor-like protein kinases (RLKs) that may be involved in the recognition of elicitors and signaling of plant responses. All contain a serine–threonine kinase domain while their extracellular domains resemble different sequence motifs. (A) Leucine-rich repeats containing Xa21 from rice. (B) Leucine-like AthLecRK1 from Arabidopsis. (C) PR protein thaumatin-like PR5K from Arabidopsis. (D) PR protein chitinase-like from tobacco. (E) Epidermal growth factor-like WAK1 from Arabidopsis. (F) Dissimilar from known sequences RLK10 from wheat. (G) Bimodal cysteine motif-exhibiting StPRKs from potato. [From Montesano *et al.* (2003) *Plant Pathol.* **4**, 73–79.]

by R proteins is indirect, i.e., through at least one-third component to which the Avr protein binds and is recognized. This implies that a correlation exists between the binding affinity of the Avr protein for the third component and the level of its HR-inducing activity. It is speculated that the third component may be a coreceptor of the Avr protein or possibly the virulence target of the Avr protein. Binding of the Avr protein to its virulence target serves as a signal to the R gene, which acts as a "guardian" of this virulence target and which then initiates the defense responses and defeat of the pathogen. However, absence of binding by the R protein will result in a lack of defense responses, leading to susceptibility of the host and victory of the pathogen. Of course, if the third component is indeed a virulence target, one would expect a correlation between the Avr proteins' contribution to virulence and its HR-inducing activity.

How Do R and *Avr* Gene Products Activate Plant Defense Responses?

It is assumed that once the R proteins recognize, directly or indirectly, the Avr proteins, they activate signaling networks that lead to resistance responses. Although several components of the signaling network have been identified, the mechanisms by which the R gene products and the so-far identified signaling components activate the host plant defense responses are still poorly understood.

The fact that R proteins share structural similarities suggests that, following recognition of the pathogen protein, the host plants use common signal transduction pathways. This is supported by the fact that resistance responses activated by various R proteins are similar. Such responses commonly include rapid ion fluxes, generation of superoxide and nitric oxide, and a hypersensitive response that includes localized cell death. It is also known that there are several signaling components that are utilized by more than one R proteins.

Some Examples of Plant Defense through R genes and their Matching *Avr* Genes

The Tomato Pto Gene

In many cases, the predicted structures of known R proteins provide some clues as to how the different protein classes may operate as receptors of *Avr* gene products and as generators and transducers of defense signals. For example, the Pto R gene of tomato, which confers resistance to the bacterium *P. syringae* pv. *tomato* (*Fig. 6-14*), codes for a cytoplasmic protein kinase that appears to interact directly with the bacterial avrPto protein that is delivered by the bacterium directly into the plant cell cytoplasm. The Pto kinase protein can interact with several other proteins, including another kinase and some that have homology to transcription factors. Some of these transcription factors possess a DNA-binding domain that recognizes a sequence present in the pro-

FIGURE 6-14 *Xanthomonas* bacteria (A) and tomato bacterial speck symptoms on tomato leaf (B) and fruit (C). (Photographs courtesy of R. J. McGovern.)

moters of genes that encode ethylene-induced defense-related proteins such as PR proteins. For example, when one of the transcription factor genes is overexpressed in a Pto R gene plant, the avrPto-mediated hypersensitive response is enhanced, which shows that the Pto protein can activate several distinct signaling pathways simultaneously. It has been shown, however, that the expression of Pto requires the presence and expression of another gene, *Prf*, which is located within the Pto gene cluster. *Prf* also encodes an LZ-NBS-LRR protein whose role in plant defense is still unknown. More recent work indicates that, perhaps, Pto is not the true R gene, but encodes the virulence target of AvrPto. The AvrPto–Pto complex is then recognized by the true R protein of the *Prf* gene, which is, presumably, "guarding' the virulence target. It appears that currently available data support an indirect recognition of AvrPto by *Prf* rather than a direct recognition of AvrPto by Pto; therefore, the interaction between AvrPto and Pto should not be considered an example of direct interaction of an Avr with its R gene but rather as interaction between an Avr protein and its virulence target.

The Tobacco N Gene

The class of cytoplasmic TIR-NBS-LRR R proteins appears in diseases caused by biotrophic fungi, bacteria, viruses, nematodes, and insects. All three domains of the N gene protein are required for proper N function. In the tobacco mosaic virus (TMV) disease (Fig. 6-15), replicase proteins of the virus confer avirulence to the virus in cultivars carrying the N gene. The N gene encodes a cytoplasmic TIR-NBS-LRR protein. The TMV genome encodes two replicase proteins, and a region of each of these proteins, which serves as the helicase of the virus, can induce a HR in tobacco carrying the N gene. The helicase function of the protein is not required for the avirulence function of the replicase. Whether recognition of the replicase protein by the N protein is direct or indirect is still unknown as is the signaling pathway for development of the defense responses. In other virus–plant combinations studied, avirulence is conferred by a portion of the viral coat protein to a host that carries matching R genes for resistance. No further information of how defense responses are triggered is available.

The Rice Pi-ta Gene

Of the fungal avr proteins, some of *Magnaporthe grisea*, the cause of rice blast on rice (Figs. 6-16A and 6-16B), and of *Cladosporium fulvum*, the cause of leaf mold on tomato, have been elucidated best. The rice blast fungus carries the avirulence gene avr-Pi-ta effective on rice cultivars carrying the resistance gene Pi-ta. Pi-ta encodes a cytoplasmic protein that contains an NBS domain and a leucine-rich carboxyl terminus. Direct interaction has been detected between the Avr-Pi-ta protein and the leucine-rich domain of Pi-ta. This is the first experimental evidence that an AVR protein interacts directly with its R protein. The predicted protease activity of AVR-Pi-ta is required for its avirulence function. How the AVR-Pita/Pi-ta interaction leads to defense responses is still unknown.

The Tomato Cf Genes

In the tomato leaf mold disease, strains of the fungus *C. fulvum* carrying any of the genes Avr2, Avr4, or Avr9 confer avirulence to tomato plants carrying the

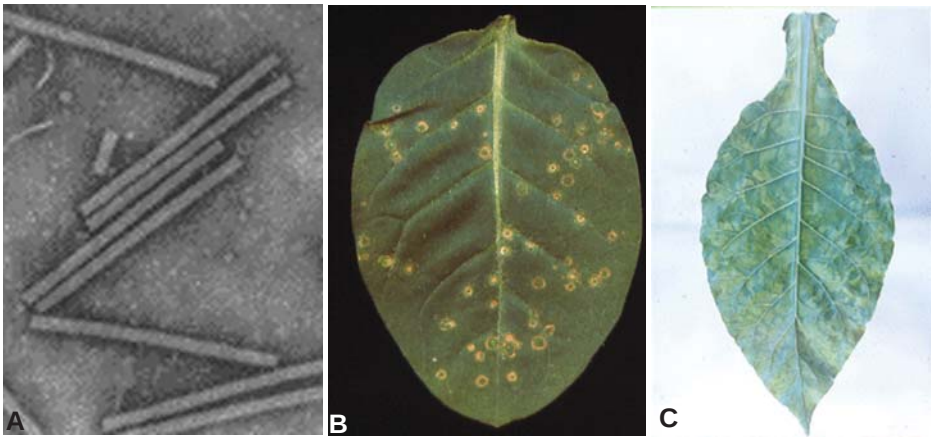


FIGURE 6-15 (A) Particles of *tobacco mosaic virus*. (B) Local lesions (hypersensitive response) on a resistant tobacco leaf. (C) Systemic mosaic symptoms on a leaf of a compatible (susceptible) tobacco plant.

FIGURE 6-16 (A) Conidia of the rice blast fungus *Magnaporthe grisea*. (B) Individual lesions and further development of rice blast on a susceptible plant. [Photographs courtesy of (A) T. E. Freeman, University of Florida, and (B) J. Kranz, University of Giessen, Germany.]

matching resistance R genes *Cf2*, *Cf4*, *or Cf9*. Avr2 encodes an extracellular cysteine-rich protein that is secreted by the fungus during growth in the apoplastic space of tomato leaves. No virulence function has been detected in the Avr2. The Cf2 protein consists of a signal peptide, an extracellular LRR region, a transmembrane region, and a short cytoplasmic tail that has no homology to known signaling motifs. The Avr2 protein is recognized by Cf2 extracellularly. Cf2 specifically requires another gene, Rcr3, in order to mediate its resistance, but Rcr3 is not required for Cf5- or Cf9-mediated resistance. As these genes are more than 90% genetically identical, they seem to activate the same defense signaling pathway after the elicitor is recognized. Thus, Rcr3 might represent the third component required for the recognition of AVR2 by Cf2. If Rcr3 indeed binds to AVR2, then Rcr3 must be at least partially extracellular. Another *C. fulvum* avirulence gene confers resistance to tomato cultivars carrying the R gene Cf9. The Cf9 R protein is localized in the plasma membrane but resembles the Cf2 R protein in most respects. The AVR9 protein, also produced in the apoplastic space of tomato leaves, encodes a protein that is processed to a 28 amino acid peptide. The AVR9 protein does not have a virulence function, but because the expression of Avr9 is induced under reduced nitrogen conditions, perhaps the gene plays a role in nitrogen metabolism of the fungus. No specific binding of the proteins of Avr9 and Cf9 genes was detected, although there is a high-affinity binding site for AVR9 in plasma membranes of tomato and other solanaceous plants. It has been suggested that perhaps these binding sites are the third component required for recognition of AVR9 by Cf9.

The Tomato Bs2 Gene

Of the other bacterial avr proteins, the AvrBs2 of *Xanthomonas campestris* pv. *vesicatoria* on pepper and several avr proteins produced by various pathovars of *Pseudomonas syringae* on their specific hosts, are the best studied so far. In the *X. campestris* pv. *vesicatoria*/pepper combination, the Bs2 codes for an NBS-LRR protein that has a hydrophobic N terminus. In addition to conferring resistance to peppers with the Bs2 R gene, the avrBs2 gene, which was shown to be highly conserved among different strains of X. *campestris* pv. *vesicatoria* and among other pathovars of *X. campestris*, is needed for full virulence of the bacterium on susceptible hosts. The avrBs2 encodes a mainly hydrophilic protein, of which the C-terminal half has homology with enzymes that synthesize or hydrolyze phosphodiester linkages, but whether this relates to its role in virulence is not known. There is a correlation between reduced virulence in susceptible hosts and in HR-inducing activity exhibited by various bacterial strains, and this may indicate indirect recognition of AvrBs2 by Bs2 after the AvrBs2 protein binds to its virulence target. Recently, however, a mutant strain was found that could not

trigger a resistant response in plants carrying Bs2 and yet it showed no reduction in its virulence in susceptible plants. Since this observation appears to uncouple the virulence and the avirulent functions of AvrBs2, it is not likely that recognition of AvrBs2 occurs after binding to its virulence target.

The Arabidopsis RPM1 Gene

The avrRpm1 gene of *P. syringae* pv. *maculicola* confers avirulence to the bacterium on pea, bean, soybean, and Arabidopsis but is also required for virulence of the same bacterium on Arabidopsis. Recognition of the AvrRpm1 in Arabidopsis requires the presence of the RPM1 gene. This gene encodes a peripheral membrane protein with LZ-NBS-LRR that probably resides at the cytoplasmic face of the plasma membrane. The RPM1 gene also confers resistance to *P. syringae* pv. *glycinea* expressing the avrB gene. The proteins encoded by avrRpm1 and avrB do not share homology except for an N-terminal eukaryotic consensus sequence for two fatty acids, myristic and palmitic. These sequences of AvrRpm1 and AvrB are required for the expression of full virulence and for localization of these proteins at the plasma membrane of the host cell. These observations suggest that AvrRpm1 and AvrB proteins are recognized by the RPM1 protein at the cytoplasmic face of the plasma membrane. It has been shown that recognition of both AvrRpm1 and AvrB by RPM1 requires the presence of RPM1-interacting protein 4 (RIN4), which is also probably localized at the plasma membrane. In the absence of RPM1, AvrRpm1 and AvrB form a complex with RIN4, which is predicted to be their virulence target, as it is a negative regulator of defense responses. These defense responses may be repressed after AvrRpm1 and AvrB bind to RIN4. In uninfected cells, RIN4 is present as a complex with RPM1. These observations support the suggestion that recognition of AvrRpm1 and AvrB by RPM1 is indirect and that the third component required for recognition is the virulence target RIN4.

The Cofunction of Two or More Genes

In many cases, expression of resistance mediated by several R proteins requires the presence of certain other genes. The proteins of these genes have the property to associate with a complex containing an ubiquitin ligase, which brings about ubiquitylation of certain other proteins. When substrate proteins become polyubiquitylated, they are targeted for degradation by the 26S proteasome. According to one theory, because the proteins targeted for degradation can be resistance regulators, degradation and removal of suspected negative regulators of resistance actually activate and set in motion the resistance responses. However, it is possible that monoubiquitylation regulates protein localization and the activity of several kinases and transcription factors and, therefore, the complex of ubiquitin with the other gene products mediates the translocation or activation of resistance regulators.

Defense Involving Bacterial Type III Effector Proteins

Most pathogenic bacteria have three types of secretion systems by which they secrete exoenzymes and other pathogenicity factors. The type I secretion system allows bacteria to secrete proteases from the cytoplasm to the extracellular space of the bacterium in a single step. Type I secretion plays a minor role in pathogenicity. The type II secretion system makes it possible for bacteria to secrete pathogenicity determinants like pectinases and cellulases and is essential for pathogenicity. The type II system employs a two-step mechanism for secretion. First, proteins are exported to the periplasm of bacteria. Then, a structure forms that spans the periplasmic compartment and the outer membrane and proteins marked by a special signal sequence are channeled through. The type II system is regulated in part by a quorum-sensing mechanism.

The type III secretion system (TTSS) consists of a set of 15 to 20 proteins associated with the bacterial cell membrane and making up the secretion apparatus that delivers or translocates host-specific "effector" proteins from the bacteria into their host plant cells (Fig. 6-17). The membrane-bound proteins are common to most kinds of bacteria that have type III secretion systems, whereas the proteins injected by them into their host cells are specific for that host plant. By translocating these bacterial "effector" proteins into their host cells, the TTSS interferes with host cell signal transduction and other cellular processes, thereby enhancing the virulence of bacteria in susceptible host cells. During delivery, a chaperon protein is bound to each "effector" protein that apparently protects the effector protein from premature interactions with other proteins. The type III secretion system occurs in all or most gram-negative pathogenic bacteria (*Erwinia*, *Pseudomonas*, *Xanthomonas*, *Ralstonia*, *Pantoea*), including those causing disease in humans and animals.

Proteins delivered into nonhost plant cells by type III secretion systems can elicit a hypersensitive response. For this reason, the TTSS is known as the hypersensitive response and pathogenicity (hrp) system. Most type III effectors from plant pathogenic bacteria were first identified as the products of typical avirulence (*avr*) genes. In bacteria, avirulence genes are defined as genes

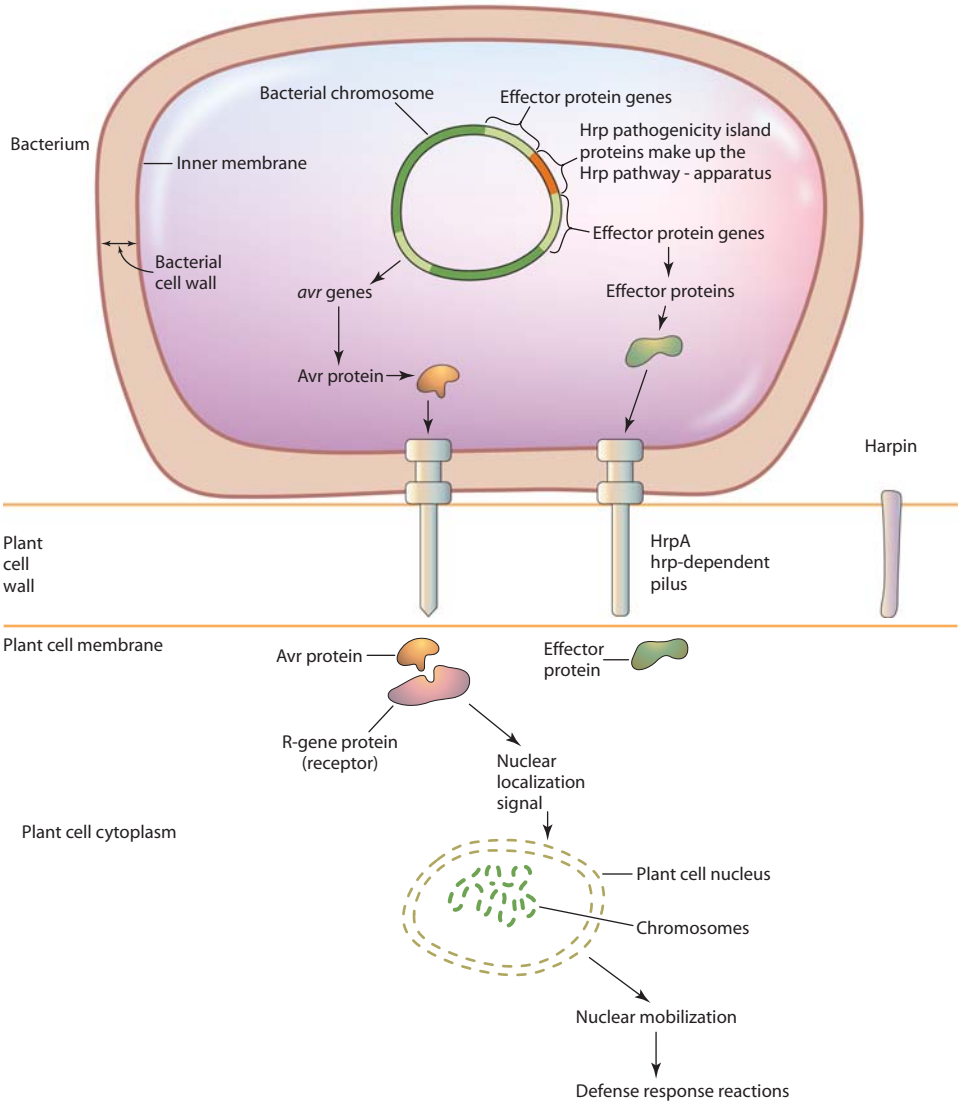


FIGURE 6-17 Diagrammatic representation of the hrp or type III secretion system in bacteria.

that can convert a normally virulent bacterial strain that infects a specific host to an avirulent one in regard to that particular host. Avirulence is usually manifested as appearance of an HR reaction on a resistant host. Because the induction of HR depends on the presence and reaction of R genes and matching hrp genes, it was thought, and later proven, that the products of hrp genes are secreted by the TTSS directly into the cytoplasm of R gene-containing host cells and lead to the induction of a hypersensitive response, i.e., cell death. It has been shown, however, that many *avr* genes that normally contribute to the defense of the host plant by being the elicitors of the HR, in the TTSS they also contribute to virulence of the bacterium by promoting more severe symptoms produced by the plant, more bacteria growing inside the leaf, and more bacteria escaping to the leaf surface. In most cases, the contribution of *avr* genes to virulence is small. Because, however, the secretion of effectors is essential for pathogenicity, it is apparent that bacteria secrete multiple effector proteins and that, therefore, they contribute to virulence in an incremental quantitative or partially redundant manner. *Xanthomonas* and *Pseudomonas* bacteria colonize the intercellular spaces (apoplast) of leaf surfaces where there are plenty of nutrients for the bacteria but where water may be a limiting factor. So, the bacteria would benefit from a susceptibility response involving leakage of water from the host cells (symplast) to the intercellular spaces (apoplast). However, the plant would benefit from a defense response, such as cell wall thickening, that would deprive the infection site from water. For the bacteria to continue to grow, they must avoid

inducing host defense responses, suppress host defense responses successfully, or both.

Harpin protein, produced by the bacterium *P. syringae* pv. *syringae*, is the hrp-dependent protein that differs from most Avr proteins in that, when injected in the leaf apoplast, it can induce a hypersensitive-like response (i.e., cell death). This implies that Harpin might function on the outside of the plant cell. The Harpin HR-like response differs from HRs induced by Avr genes in that it does not depend on a matching R gene. Harpin can associate with liposomes and with bilayer membranes on which it apparently forms pores; through the pores, then, water and nutrients move out of the cell to the apoplast or, more likely, the pores serve as openings so that other types of effectors can be translocated into the cells.

Several *avr* genes have been implicated in the suppression of host defenses. Thus, the *P. syringae* pv. *phaseolicola RW60* strain can be converted by the *avrPphF* gene from avirulent to virulent on a particular bean cultivar (A) in which it suppresses the hypersensitive response. Interestingly, the same *avr* gene in the same bacterial strain (RW60) increases the HR induced by RW60 in another bean cultivar (B). This enhancement of the HR by the first *avr* gene can be suppressed by another *avr* gene. Because the suppression of HR by these genes is host specific, this points to a molecular "arms race" between bacterial effectors and host targets. It appears that the target of one effector (e.g., AvrPphC) is the R gene product, which will detect a second effector (e.g., AvrPphF). Other examples of suppression of defense responses by type III effectors are known, suggesting that such genes may interfere with the induction of defense responses at a level similar to the infection by the avirulent and the virulent pathogens.

Active Oxygen Species, Lipoxygenases, and Disruption of Cell Membranes

The plant cell membrane consists of a phospholipid bilayer in which many kinds (Figure 5-2) of protein and glycoprotein molecules are embedded. The protein molecules are often organized in groups, some of which form channels on the membrane and allow ions and metabolites to enter and exit the cell. The cell membrane in the form of endoplasmic reticulum and organelles compartmentalizes the cell into areas in which specific compounds are kept separated from others and certain biochemical reactions take place. In addition, the cell membrane is an active site for the induction of defense mechanisms; e.g., it serves as the anchor of R gene-coded proteins that recognize the elicitors released by the pathogen and subsequently trigger the hypersensitive response.

The attack of cells by pathogens, or exposure to pathogen toxins and enzymes, often results in structural and permeability changes of the cell membrane. These changes are generally thought to be an expression of susceptibility and disease development. In many host–pathogen combinations, however, particularly those involving the hypersensitive response, some membrane changes play a role in the defense against invasion by the pathogen. The most important membrane-associated defense responses include (1) the release of molecules important in signal transduction within and around the cell and, possibly, systemically through the plant; (2) the release and accumulation of reactive oxygen "species" and of lipoxygenase enzymes; and (3) as a result of the loss of compartmentalization, activation of phenol oxidases and oxidation of phenolics (Figures 4-13, 6-11).

In many host–fungus interactions, one of the first events detected in attacked host cells, or cells treated artificially with fungal elicitors, is the rapid and transient generation of activated oxygen species, including superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH). The generation of superoxide and of other reactive oxygen species as defense response happens most dramatically in localized infections, but it also occurs in general and systemic infections, as well as in plants treated with chemicals that induce systemic acquired resistance. These highly reactive oxygen species are thought to be released by the multisubunit NADPH oxidase enzyme complex of the host cell plasma membrane. They appear to be released in affected cells within seconds or minutes from contact of the cell with the fungus or its elicitors and reach a maximum activity within minutes to a few hours.

The activated oxygen species trigger the hydroperoxidation of membrane phospholipids, producing mixtures of lipid hydroperoxides. The latter are toxic, their production disrupts the plant cell membranes, and they seem to be involved in normal or HR-induced cell collapse and death. Active oxygen species may also be involved in host defense reactions through the oxidation of phenolic compounds into more toxic quinones and into lignin-like compounds. The presence of active oxygen species, however, also affects the membranes and the cells of the advancing pathogen either directly or indirectly through the hypersensitive response of the host cell. The production of reactive oxygen species in affected but surviving nearby cells is kept under control by the radical scavenger enzymes superoxide dismutase, catalase, ascorbate peroxidase, etc. Several isoenzymes of each of these are produced, with different ones of them appearing at different stages after inoculation.

The oxygenation of membrane lipids seems to involve various lipoxygenases as well. These are enzymes that catalyze the hydroperoxidation of unsaturated fatty acids, such as linoleic acid and linolenic acid, which

have been released previously from membranes by phospholipases. The lipoxygenase-generated hydroperoxides formed from such fatty acids, in addition to disrupting the cell membranes and leading to HR-induced cell collapse of host and pathogen, are also converted by the cell into several biologically active molecules, such as jasmonic acid, that play a role in the response of plants to wounding and other stresses. Jasmonic acid, for example, which is the precursor of the wound hormone traumatin, appears to induce numerous protein changes and acts as a signal transducer of the defense reaction in plant–pathogen interactions.

Reinforcement of Host Cell Walls with Strengthening Molecules

In several plant diseases caused by fungi, the walls of cells that come in contact with the fungus produce, modify, or accumulate several defense-related substances that reinforce the resistance of the wall to invasion by the pathogen. Among the defensive substances produced or deposited in plant cell walls being invaded by fungi are callose, glycoproteins such as extensin that are rich in the amino acid hydroxyproline, phenolic compounds of varying complexity including lignin and suberin, and mineral elements such as silicon and calcium. Some of these substances are also produced or deposited in defensive cell wall structures such as the papillae. Many of these substances form complex polymers and also react and cross-link with one another, thereby forming more or less insoluble cell wall structures that confine the invading fungus and prevent the further development of disease. Of course, in cases in which the host lacks resistance or exhibits incomplete resistance, apparently the host, with or without interference by fungal secretions, fails to produce reinforcing compounds or produces them too slowly to be effective and the fungus manages to invade the cell.

Production of Antimicrobial Substances in Attacked Host Cells

Pathogenesis-Related Proteins

Pathogenesis-related proteins, often called PR proteins, are a structurally diverse group of plant proteins that are toxic to invading fungal pathogens. They are widely distributed in plants in trace amounts, but are produced in much greater concentration following pathogen attack or stress. PR proteins exist in plant cells intracellularly and also in the intercellular spaces, particularly in the cell walls of different tissues. Varying types of PR proteins have been isolated from each of several crop plants. Different plant organs, e.g., leaves, seeds, and roots, may produce different sets of PR proteins. Different PR proteins appear to be expressed differentially in their hosts in the field when temperatures become stressful, low or high, for extended periods.

The several groups of PR proteins have been classified according to their function, serological relationship, amino acid sequence, molecular weight, and certain other properties. PR proteins are either extremely acidic or extremely basic and therefore are highly soluble and reactive. At least 14 families of PR proteins are recognized. The better known PR proteins are PR1 proteins (antioomycete and antifungal), PR2 (β-1,3-glucanases), PR3 (chitinases), PR4 proteins (antifungal), PR6 (proteinase inhibitors) (Fig. 6-19), thaumatine-like proteins, defensins, thionins, lysozymes, osmotinlike proteins, lipoxygenases, cysteine-rich proteins, glycine-rich proteins, proteinases, chitosanases, and peroxidases. There are often numerous isoforms of each PR protein in various host plants.

Although healthy plants may contain trace amounts of several PR proteins, attack by pathogens, treatment with elicitors, wounding, or stress induce transcription of a battery of genes that code for PR proteins (Fig. 6-18). This occurs as part of a massive switch in the overall pattern of gene expression, during which normal protein production nearly ceases. The signal compounds responsible for induction of PR proteins include salicylic acid, ethylene, xylanase, the polypeptide systemin, jasmonic acid, and probably others (Fig. 6-11).

The significance of PR proteins lies in the fact that they show strong antifungal and other antimicrobial activity (Figure 6-19). Some of them inhibit spore release and germination, whereas others are associated with strengthening of the host cell wall and its outgrowths and papillae. Some of the PR proteins, e.g., β-1,3-glucanase and chitinase, diffuse toward and affect (break down) the chitin-supported structure of the cell walls of several but not all plant pathogenic fungi, whereas lysozymes degrade the glucosamine and muramic acid components of bacterial cell walls. Lipoxygenases and lipid peroxidases generate antimicrobial metabolites as well as secondary signal molecules such as jasmonic acid. Structurally similar defensins also occur in mammals, birds, and insects. Plant defensins, which are basic cysteine-rich peptides, have antimicrobial activity and accumulate through the ethylene and jasmonic acid pathway. Plants genetically engineered to express chitinase genes show good resistance against the soilborne fungus *Rhizoctonia solani*. Tobacco plants treated with lipopolysaccharides obtained from the outer wall of gram-negative bacteria produced several PR proteins and exhibited enhanced defense responses in tobacco against *Phytophthora nicotianae*, including the production of a systemic response in the leaves of plants inoculated through the roots. Signal molecules that induce PR protein synthesis seem to be transported systemically to other parts of the

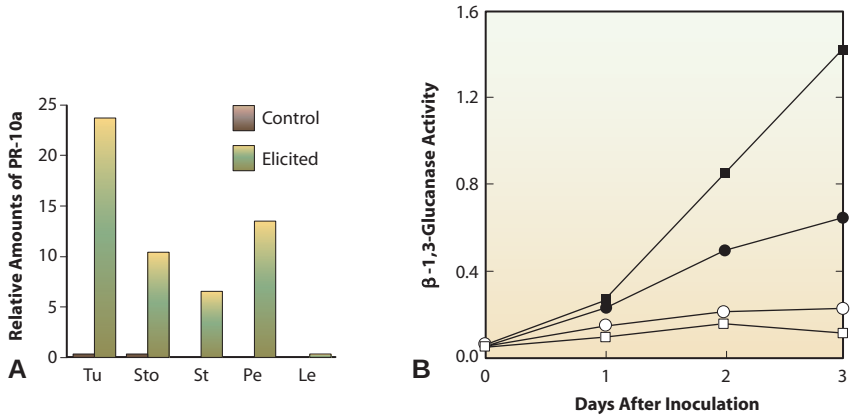


FIGURE 6-18 (A) Production and accumulation of a pathogenesis-related protein (PR10a) in potato tissues either untreated (control) or elicited by treating cut surfaces with a homogenate of the late blight fungus *Phytophthora infestans* and incubating for 4 days. Tu, tuber; Sto, stolon; St, stem; Pe, petiole; Le, leaf. [From Constabel and Brisson (1995). *Mol. Plant-Microbe Interact.* 8, 104–113.] (B) Levels of activity of the antifungal protein β-1,3-glucanase in the intercellular fluid of barley leaves, either left uninoculated (□, ○) or inoculated with the powdery mildew fungus *Erysiphe graminis* f. sp. *hordei* (■, ●). The two barley varieties are nearly isogenic, except that one (□, ■) carries an additional resistance gene that makes it resistant, whereas the other (○, ●) is susceptible. [From Jutidamrongphan *et al.* (1991). *Mol. Plant-Microbe Interact.* **4**, 234–238.]

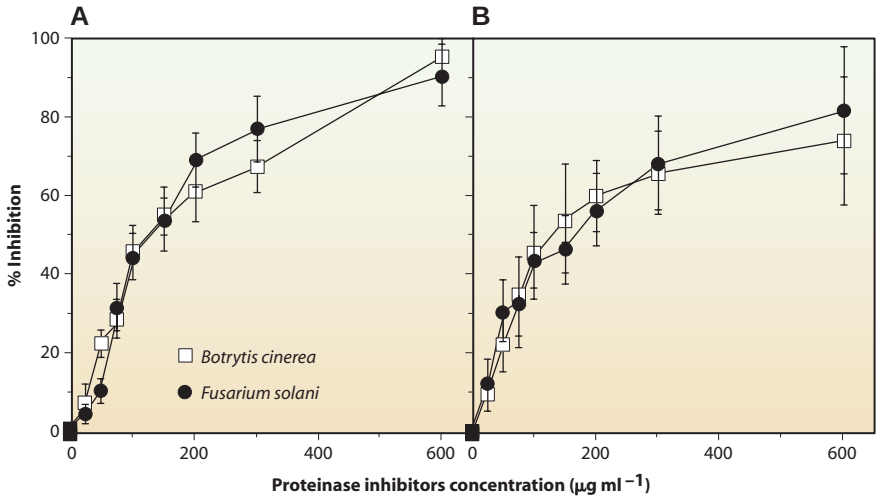


FIGURE 6-19 Inhibition of (A) spore germination and (B) germ tube elongation of fungi *Botrytis cinerea* and *Fusarium solani*, which do not infect cabbage, by proteinase inhibitors obtained from young cabbage leaves. The inhibitors caused leakage of the cellular contents of these fungi. The cabbage fungal pathogen *Alternaria brassicicola* was not affected by these proteinase inhibitors. [From Lorito *et al.* (1994). *Mol. Plant-Microbe Interact.* 7, 525–527.]

plant and to reduce disease initiation and intensity in those parts for several days or even weeks.

Defense through Production of Secondary Metabolites: Phenolics

Simple Phenolic Compounds

It has often been observed that certain common phenolic compounds that are toxic to pathogens are produced and accumulate at a faster rate after infection, especially in a resistant variety of plant relative to a susceptible variety. Chlorogenic acid, caffeic acid, and ferulic acid are examples of such phenolic compounds (Fig. 6-20). In peach, chlorogenic acid is present in quite high concentration both in immature fruit and in fruit of varieties resistant to the brown rot disease caused by the fungus *Monilinia fructicola*. The fruit is resistant in both cases, not because of the toxicity of the acid

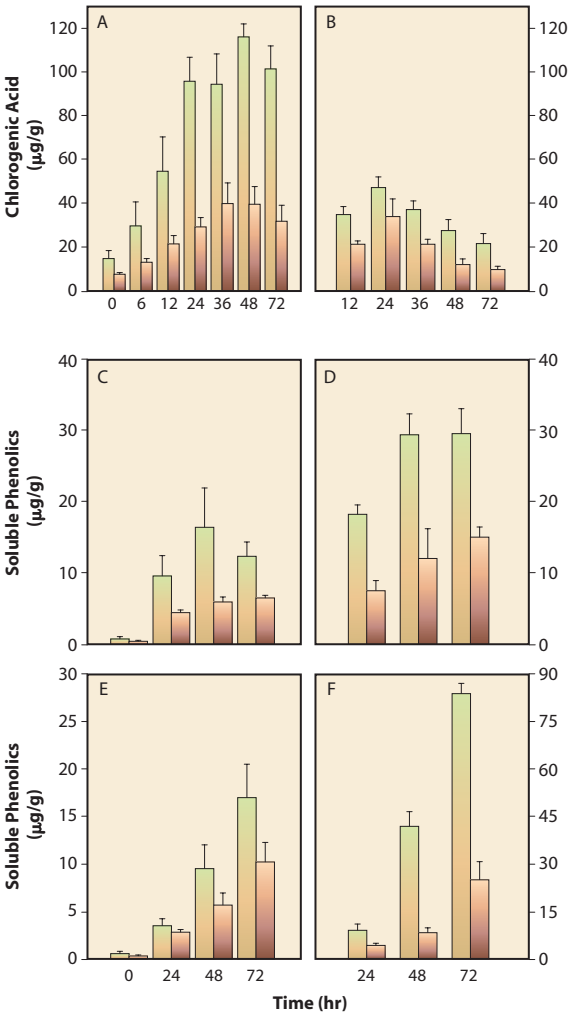


FIGURE 6-20 Production of chlorogenic acid and other soluble and wall-bound phenolics in normal (white bars) and transgenic (dark bars) potato tubers after wounding (A, C, and E) and after spraying with arachidonic acid, an elicitor of the hypersensitive defense response (B, D, and F). Transgenic plants produced an enzyme that inactivates tryptophan, a precursor of phenolics and lignin. Chlorogenic acid was increased by wounding but not by elicitation. Soluble and wall-bound phenolics increased after wounding and even more following treatment with the elicitor, but the increase was smaller in the transgenic tubers (dark bars) than in the normal tubers. Accordingly, the transgenic tubers in these treatments were more susceptible to infection when inoculated with zoospores of *Phytophthora infestans* than the treated normal plants. [From Yao *et al.* (1995). *Plant Cell* 7, 1787–1799.]

to the causal fungus, but rather because it inhibits the production of fungal enzymes that cause degradation of host tissue. In date palm tree roots, cell wall-bound hydroxybenzoic acid and sinapic acid increased 11–12 times as much in cultivars resistant to *Fusarium* than they did in susceptible cultivars. In plants such as vetch (*Vicia sativa*), resistance to the higher parasitic plant *Orobanche aegyptiaca* appears to result from higher levels of free and bound phenolics, lignin and peroxidase activity produced in the roots of resistant varieties following infection, compared to susceptible ones. In cacao infected with the witches' broom fungus *Crinipellis perniciosa*, infected young stems contain 7–8 times as much caffeine, which inhibits growth of the fungus in culture, than healthy stems. In another polygenic disease, the black sigatoka disease of banana caused by the fungus *Mycosphaerella fijiensis*, plant defenses included an activation of phenylalanine ammonia lyase and a subsequent accumulation of phenolic compounds. It also caused early activation of a banana response to the fungal compound trihydroxytetralone (THT), which, in resistant varieties, caused necrotic microlesions and elicitation of infection-induced defense reactions leading to incompatibility (resistance) between the pathogen and the host plant. In susceptible varieties, however, the fungus produced necrotizing levels of THT only at the later stages of pathogenesis after a compatible interaction had been established and typical symptoms had developed. Although some of the common phenolics may each reach concentrations that could be toxic to the pathogen, it should be noted that several of them appear concurrently in the same diseased tissue, and it is possible that the combined effect of all fungitoxic phenolics present, rather than that of each one separately, is responsible for the inhibition of infection in resistant varieties. It has even been proposed that because of the universal uniform or strategic location of phenolics-storing plant cells, these cells can, by decompartmentation and rapid oxidation of their phenolic contents, self-sacrifice, leading to the first line of defense — cell death — or leading to the production of a slower defense line — a peridermal defense layer.

Toxic Phenolics from Nontoxic Phenolic Glycosides

Many plants contain nontoxic glycosides, i.e., compounds consisting of a sugar (such as glucose) joined to another, often phenolic, molecule. Several fungi and bacteria are known to produce or to liberate from plant tissues the enzyme glycosidase that can hydrolyze such complex molecules and release the phenolic compound from the complex. Some of the released phenolics are quite toxic to the pathogen, especially after further oxidation, and appear to play a role in the defense of the plant against infection.

Role of Phenol-Oxidizing Enzymes in Disease Resistance

The activity of many phenol-oxidizing enzymes (polyphenol oxidases) is generally higher in the infected

tissue of resistant varieties than in infected susceptible ones or in uninfected healthy plants. The importance of polyphenol oxidase activity in disease resistance probably stems from its property to oxidize phenolic compounds to quinones, which are often more toxic to microorganisms than the original phenols. It is reasonable to assume that an increased activity of polyphenol oxidases will result in higher concentrations of toxic products of oxidation and therefore in greater degrees of resistance to infection. A complex interaction occurs during fruit ripening in which levels of lipoxygenases increase and break down diene, a compound that is present in young, immature fruit and is toxic to fungi. These events normally result in infection (loss of resistance) of the ripening fruit. In some fruit, however, elicitors from nonpathogenic fungi stimulate production of the phenolic compound epicatechin, which inhibits the activity of lipoxygenases. As a result, epicatechin decreases degradation of the antifungal diene, thereby preventing decay of the ripening fruit by anthracnose fungi.

Another phenol oxidase enzyme, peroxidase, both oxidizes phenolics to quinones and generates hydrogen peroxide. The latter not only is antimicrobial in itself, but it also releases highly reactive free radicals and in that way further increases the rate of polymerization of phenolic compounds into lignin-like substances. These substances are then deposited in cell walls and papillae and interfere with the further growth and development of the pathogen.

Phytoalexins

Phytoalexins are toxic antimicrobial substances produced in appreciable amounts in plants only after stimulation by various types of phytopathogenic microorganisms or by chemical and mechanical injury. Phytoalexins are produced by healthy cells adjacent to localized damaged and necrotic cells in response to materials diffusing from the damaged cells. Phytoalexins are not produced during compatible biotrophic infections. Phytoalexins accumulate around both resistant and susceptible necrotic tissues. Resistance occurs when one or more phytoalexins reach a concentration sufficient to restrict pathogen development. Most known phytoalexins are toxic to and inhibit the growth of fungi pathogenic to plants, but some are also toxic to bacteria, nematodes, and other organisms. More than 300 chemicals with phytoalexinlike properties have been isolated from plants belonging to more than 30 families. The chemical structures of phytoalexins produced by plants of a family are usually quite similar; e.g., in most legumes, phytoalexins are isoflavonoids, and in the Solanaceae they are terpenoids. Most of the phytoalexins are produced in plants in response to infection by fungi, but a few bacteria, viruses, and nematodes have also been shown to induce the production of phytoalexins. Some of the better studied phytoalexins include phaseollin in bean (Fig. 6-21); pisatin in pea; glyceollin in soybean, alfalfa, and clover; rishitin in potato; gossypol in cotton; and capsidiol in pepper.

Phytoalexin production and accumulation occur in healthy plant cells surrounding wounded or infected cells and are stimulated by alarm substances produced and released by the damaged cells and diffusing into the adjacent healthy cells. Most phytoalexin elicitors are generally high molecular weight substances that are constituents of the fungal cell wall, such as glucans, chitosan, glycoproteins, and polysaccharides. The elicitor molecules are released from the fungal cell wall by host plant enzymes. Most such elicitors are nonspecific, i.e., they are present in both compatible and incompatible races of the pathogen and induce phytoalexin accumulation irrespective of the plant cultivar. A few phytoalexin elicitors, however, are specific, as the accumulation of phytoalexin they cause on certain compatible and incompatible cultivars parallels the phytoalexin accumulation caused by the pathogen races themselves. Although most phytoalexin elicitors are thought to be of pathogen origin, some elicitors, e.g., oligomers of galacturonic acid, are produced by plant cells in response to infection or are released from plant cell walls after their partial breakdown by cell wall-degrading enzymes of the pathogen.

The formation of phytoalexins in a susceptible (compatible) host following infection by a pathogen seems, in some cases, to be prevented by suppressor molecules produced by the pathogen. The suppressors seem to also be glucans or glycoproteins, or one of the toxins produced by the pathogen.

The mechanisms by which phytoalexin elicitors, phytoalexin production, phytoalexin suppressors, genes for resistance or susceptibility, and the expression of resistance or susceptibility are connected are still not well understood. Several hypotheses have been proposed to explain the interconnection of these factors, but much more work is needed before a satisfactory explanation can be obtained.

Species or races of fungi pathogenic to a particular plant species seem to stimulate the production of generally lower concentrations of phytoalexins than nonpathogens. For example, in the case of pisatin production by pea pods inoculated with the pathogen *Ascochyta pisi*, pea varieties produce concentrations of pisatin that are approximately proportional to the resistance of the variety to the pathogen. When the same pea variety is inoculated with different strains of the fungus, the concentration of pisatin produced is approximately

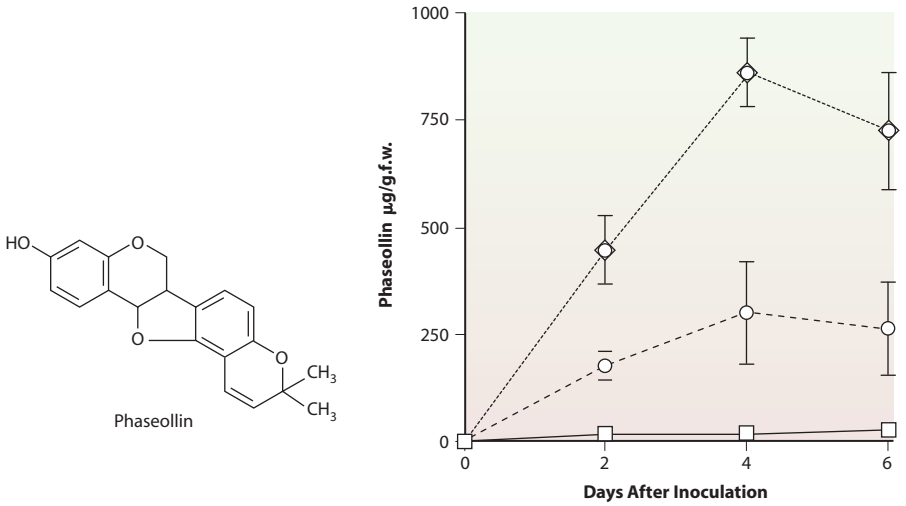


FIGURE 6-21 Levels of the phytoalexin phaseollin produced at infection sites in bean pods following inoculation with three races of the halo blight bacterium *Pseudomonas syringae* pv. *phaseolicola*. Virulent race 6 (□) infects without causing a defense response nor production of the phytoalexin. The same race 6 was transformed with an avirulence gene corresponding to resistance gene R2 (○) and with an avirulence gene to R3 (◇), and the transformants induced visibly different hypersensitive responses and also different levels of phytoalexin. [From Mansfield *et al.* (1994). *Mol. Plant-Microbe Interact.* **6**, 726–739.]

inversely proportional to the virulence of each particular fungal strain inoculated on the pea variety. Also, in soybean plants infected with the fungus *Phytophthora megasperma* f. sp. *glycinea*, inoculations of fungal races on incompatible host cultivars resulted in earlier accumulations and higher concentrations of the phytoalexin glyceollin than inoculations of fungal races on compatible cultivars. It has been suggested that the higher concentrations of glyceollin in incompatible host–pathogen combinations are the result of reduced biodegradation rather than increased biosynthesis of the phytoalexin. In some host-pathogen systems, however, e.g., in the bean/*Colletotrichum lindemuthianum* and the potato/*Phytophthora infestans* systems, the respective phytoalexins, such as phaseollin and rishitin, reach equal or higher concentrations in compatible (susceptible) hosts compared to incompatible (resistant) ones.

However, pathogenic races or species of fungi seem to be less sensitive to the toxicity of the phytoalexin(s) produced by their host plant than nonpathogenic fungi. It has been suggested that pathogens may have an adoptive tolerance mechanism that enables them to withstand higher concentrations of the host phytoalexin after earlier exposures to lower concentrations of the phytoalexin. It is known, however, that many pathogenic fungi can metabolize the host phytoalexin into a nontoxic compound, thereby decreasing the toxicity of the phytoalexin to the pathogen. It is also known that numerous pathogenic fungi are successful in causing disease, although they are sensitive to or unable to metabolize the host phytoalexins. Furthermore, some fungi that can either degrade or tolerate certain phytoalexins are unable to infect the plants that produce them.

In general, it appears that phytoalexins may play a decisive or an auxiliary role in the defense of some hosts against certain pathogens, but their significance, if any, as factors of disease resistance in most host–pathogen combinations is still unknown.

DET\OXIFICATION OF PATHOGEN TOXINS BY PLANTS

In at least some of the diseases in which the pathogen produces a toxin, resistance to disease is apparently the same as resistance to the toxin. Detoxification of at least some toxins, e.g., HC toxin and pyricularin, produced by the fungi *Cochliobolus carbonum* and *Magnaporthe grisea*, respectively, is known to occur in plants and may play a role in disease resistance. Some of these toxins appear to be metabolized more rapidly by resistant varieties or are combined with other substances and form less toxic or nontoxic compounds. The amount of the nontoxic compound formed is often proportional to the disease resistance of the variety.

Resistant plants and nonhosts are not affected by the specific toxins produced by *Cochliobolus*, *Periconia*, and *Alternaria*, but it is not yet known whether the selective action of these toxins depends on the presence of receptor sites in susceptible but not in resistant vari-

eties, on detoxification of the toxins in resistant plants, or on some other mechanism.

IMMUNIZATION OF PLANTS AGAINST PATHOGENS

Defense through Plantibodies

In humans and animals, defenses against pathogens are often activated by natural or artificial immunization, i.e., by a subminimal natural infection with the pathogen or by an artificial injection of pathogen proteins and other antigenic substances. Both events result in the production of antibodies against the pathogen and, thereby, in subsequent prolonged protection (immunity) of the human or animal from infection by any later attacks of the pathogen.

Plants, of course, do not have an immune system like that of humans and animals, i.e., they do not produce antibodies. In the early 1990s, however, transgenic plants were produced that were genetically engineered to incorporate in their genome, and to express, foreign genes, such as mouse genes that produce antibodies against certain plant pathogens. Such antibodies, encoded by animal genes but produced in and by the plant, are called plantibodies. It has already been shown that transgenic plants producing plantibodies against coat proteins of viruses, e.g., *artichoke mottle crinkle virus*, to which they are susceptible, can defend themselves and show some resistance to infection by these viruses. It is expected that, in the future, this type of plant immunization will yield dividends by expressing animal antibody genes in plants that will produce antibodies directed against specific essential proteins of the pathogen, such as viral coat proteins and replicase or movement proteins, and fungal and bacterial enzymes of attack.

Whole antibodies or fragments of antibodies can be expressed easily in plants following integration of a transgene into the plant genome, or by transient expression of the gene using viral vectors, infiltration of the gene by *Agrobacterium*, or through biolistics. Plants such as tobacco, potato, and pea have been shown to be good producers of antibody for pharmaceutical purposes. Plants have been shown to produce functional antibodies that can be used to increase the resistance of plants against specific pathogens. So far, functional plantibodies, produced by plants against specific plant pathogens, that have been shown to increase the resistance of the host plant to that pathogen include the following: Plantibodies to *tobacco mosaic virus* in tobacco decreased infectivity of the virus by 90%; to *beet necrotic yellow vein virus*, also in tobacco, provides a partial protection against the virus in the early stages of infection and against development of symptoms later on; to stolbur phytoplasma and to corn stunt spiroplasma, also in tobacco, which remained free from infection for more than two months. However, attempts to engineer plantibody-mediated resistance to plant parasitic nematodes have been unsuccessful so far. Generally, however, the expression of complete or fragment antibodies in plants has been only partially effective or mostly ineffective so far. Plantibody-derived resistance appears mostly as a delay in the development of disease and, barring a breakthrough, it does not appear that it will become an effective means of plant disease control in the near future.

Resistance through Prior Exposure to Mutants of Reduced Pathogenicity

Inoculation of avocado fruit with a genetically engineered, reduced pathogenicity strain of the anthracnose fungus *Colletotrichum gloeosporioides*, which does produce an appressorium, results in delayed decay of the fruit. Such an inoculation brings about increased levels of biochemical defense indicators, such as H^+-ATPase activity, reactive oxygen species, phenylalanine ammonia lyase, the natural antioxidant phenol epicatechin, the antifungal compound diene, and eventual fruit resistance with delay of fruit decay. However, inoculation of fruit with a similar mutant strain that does not produce an appressorium causes no activation of early signaling events and no fruit resistance. It would appear that initiation of the early signaling events that affect fruit resistance depends on the ability of the pathogen to interact with the fruit and initiate its defense mechanisms during appressorium formation.

SYSTEMIC ACQUIRED RESISTANCE

Induction of Plant Defenses by Artificial Inoculation with Microbes or by Treatment with Chemicals

As discussed earlier, plants do not naturally produce antibodies against their pathogens, and most of their biochemical defenses are inactive until they are mobilized by some signal transmitted from an attacking pathogen. It has been known for many years, however, that plants develop a generalized resistance in response to infection by a pathogen or to treatment with certain natural or synthetic chemical compounds.

Induced resistance is at first localized around the point of plant necrosis caused by infection by the pathogen or by the chemical, and it is then called local

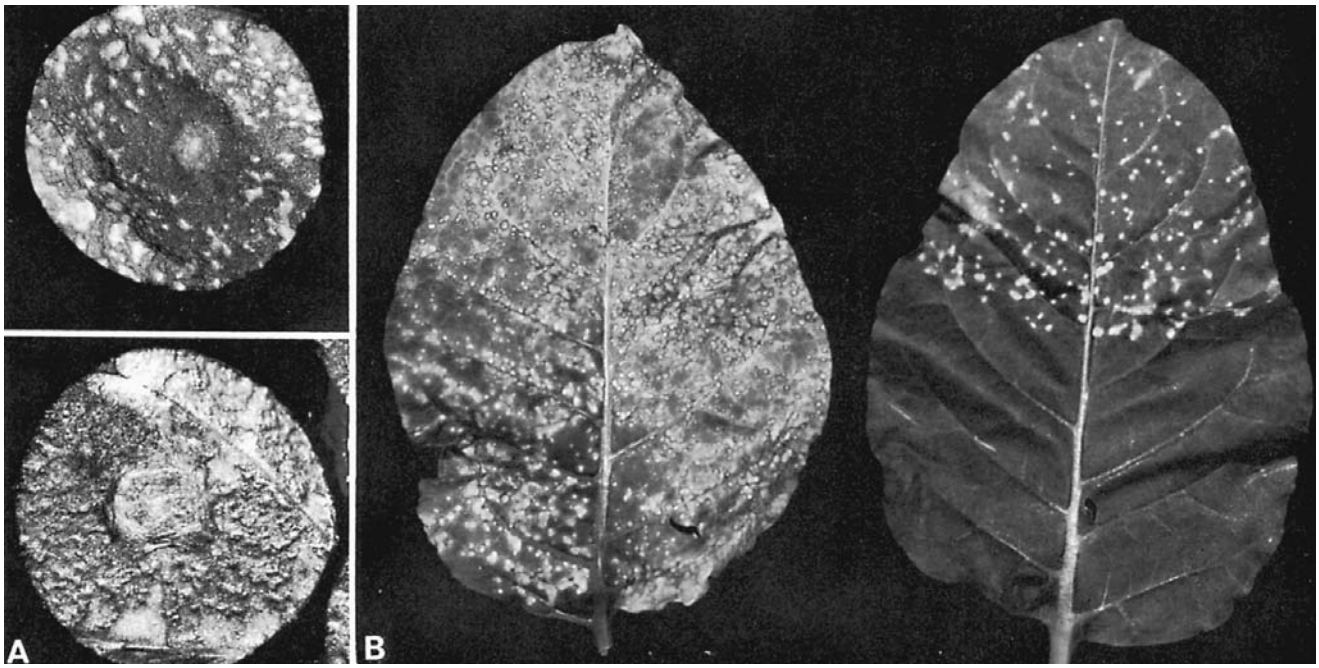


FIGURE 6-22 (A) Development of local acquired resistance to tobacco mosaic virus (TMV) around a local lesion caused by the same virus on a resistant tobacco variety. When the same leaves were reinoculated with TMV seven days later, no new lesions formed near the original one because of local acquired resistance (top), but when they were reinoculated with a different virus, no zone free of lesions remained (bottom). (B) The upper (tip) half of the leaf at the right was inoculated with TMV, and seven days later both leaves were inoculated with the same virus over their entire surface. The leaf at the left developed numerous local lesions throughout, whereas the previously half-inoculated leaf at the right developed almost no additional lesions because of acquired local and systemic resistance. [From Ross (1961). *Virology* **14**, 329–339 and 340–358.]

acquired resistance (Fig. 6-22A). Subsequently, resistance spreads systemically and develops in distal, untreated parts of the plant and is called systemic acquired resistance (Fig. 6-22B). It is known now that several chemical compounds, e.g., salicylic acid, arachidonic acid, and 2,6-dichloroisonicotinic acid, may induce localized and systemic resistance in plants at levels not causing tissue necrosis. Jasmonic acid is another type of compound, derived primarily from oxidation of fatty acids, that leads to systemic acquired resistance, often in cooperation with salicylic acid and ethylene, leading to the production of defensins. Probenazole, a synthetic chemical used in Asia for the control of rice blast disease caused by the fungus *Magnaporthe grisea*, has been shown to act upstream from the salicylic acid transcribing gene and, thereby, causing accumulation of salicylic acid. Probenazole induces systemic acquired resistance in rice against rice blast, in tomato against the bacterial pathogen *P. syringae* pv. *tabaci*, and in tobacco against the *tobacco mosaic virus*. Similarly, riboflavin was shown to induce systemic acquired resistance but it activates it in a distinct manner not involving salicylic acid. Such chemicals may be effective in inducing resistance in plants when they are applied through the roots, as a foliar spray (Fig. 6-23), or by stem injection. Local acquired resistance is induced, for example, in a 1 to 2 mm zone around local lesions caused by tobacco mosaic virus on hypersensitive tobacco varieties and probably in other host–pathogen combinations. Local acquired resistance results in near absence of lesions immediately next to the existing lesion and in smaller and fewer local lesions developing farther out from the existing local lesions when inoculations are made at least 2–3 days after the primary infection. Local acquired resistance may play a role in natural infections by limiting the number and size of lesions per leaf unit area.

Systemic acquired resistance acts nonspecifically throughout the plant and reduces the severity of disease caused by all classes of pathogens, including normally virulent ones. It has been observed in many dicot and monocot plants, but has been studied most in cucurbits, solanaceous plants, legumes, and gramineous plants following infection with appropriate fungi, bacteria, and viruses. Systemic acquired resistance is certainly produced in plants following expression of the hypersensitive response (Fig. 6-24). Localized infections of young plants, e.g., cucumber with a fungus (*Colletotrichum lagenarium*), a bacterium (*Pseudomonas lachrymans*), or a virus (*tobacco necrosis virus*), lead within a few

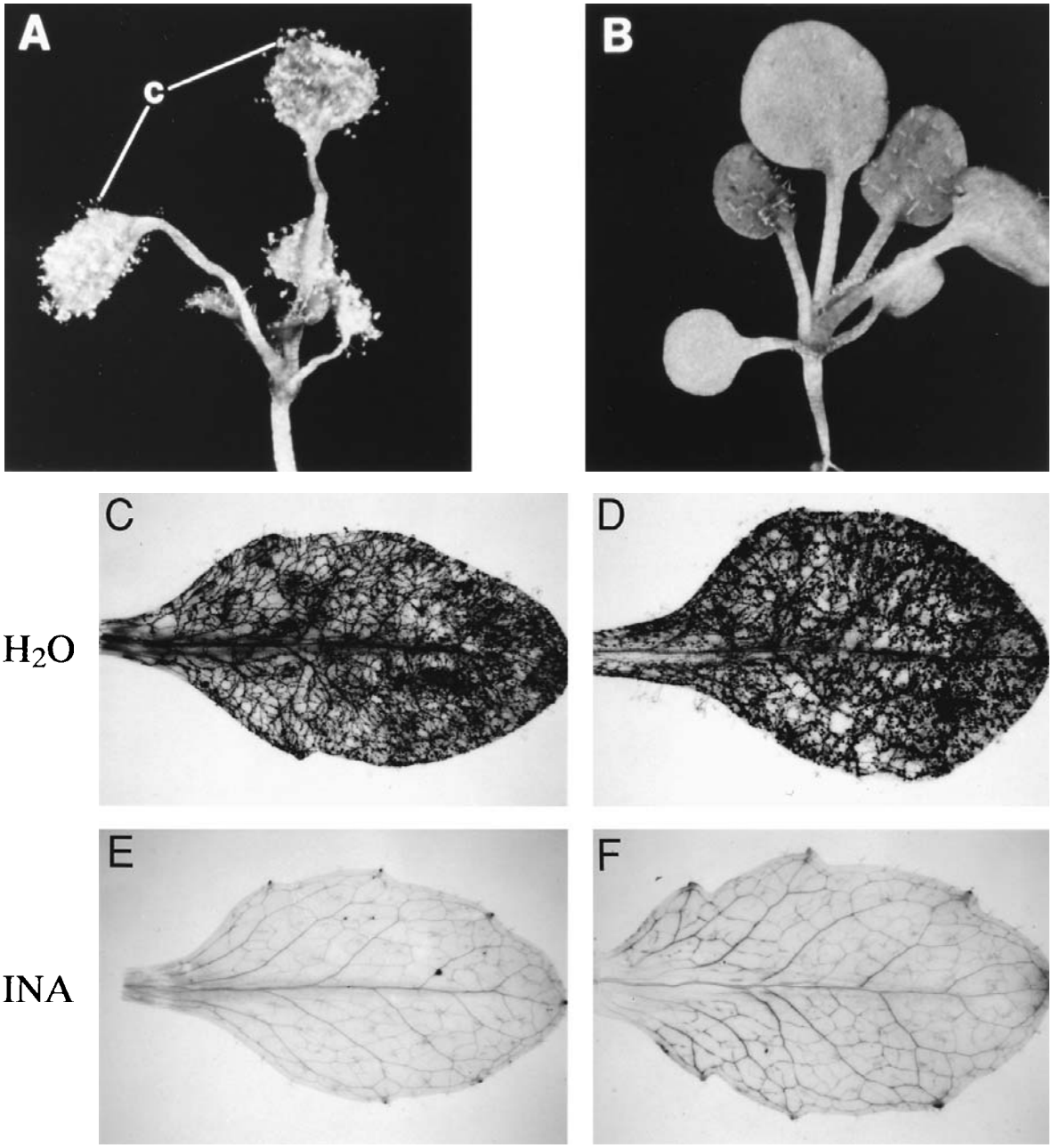


FIGURE 6-23 Induced resistance in *Arabidopsis* plants sprayed with water (A, C, D), salicylic acid (B), or 2,6-dichloroisonicotinic acid (INA) and inoculated with spores of *Peronospora parasitica* five (A, B) or four (C–F) days later. At six (A, B) or ten (C–F) days after inoculation, individual leaves revealed numerous oomycete structures in heavily infected H_2O-treated leaves and almost no oomycete structures in INA-treated leaves. Plants in A–C and E are normal, whereas those in D and F were transformed with a gene that blocks the accumulation of salicylic acid, indicating that INA can induce resistance in the absence of salicylic acid accumulation. C, conidiophores. [Photographs courtesy of J. A. Ryals, Ciba Agric. Biotechnology. A and B from Uknes *et al*., *Mol. Plant-Microbe Interact*. **6**, 692–698; C–F from Vernooij *et al*. (1995). *Mol. Plant-Microbe Interact*. **8**, 228–234.]

days' time to broad-spectrum, systemic acquired resistance to at least 13 diseases caused by fungi, bacteria, and viruses. A single inducing infection protects cucumber from all pathogens tested for 4 to 6 weeks; when a second, booster inoculation is made 2 to 3 weeks after the primary infection, the plant acquires season-long resistance to all tested pathogens. The degree of systemic acquired resistance seems to correlate well with the number of lesions produced on the induced leaf until a saturation point is reached. Systemic acquired resistance, however, cannot be induced after the onset of flowering and fruiting in the host plant.

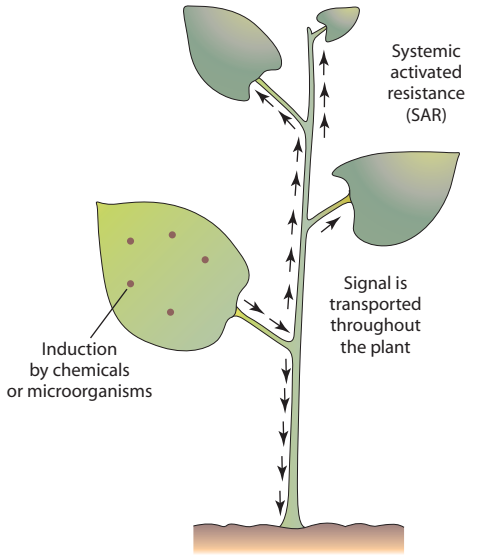


FIGURE 6-24 Principle of systemic activated (or acquired) resistance. A leaf treated with certain chemicals or with pathogens causing necrotic lesions produces a signal compound(s) that is transported systemically throughout the plant and activates its defense mechanisms, making the entire plant resistant to subsequent infections.

Systemic acquired resistance is characterized by the coordinate induction in uninfected leaves of inoculated plants of at least nine families of genes now known as systemic acquired resistance genes. Products of several SAR genes, e.g., β-1,3-glucanases, chitinases, cysteine-rich proteins related to thaumatin, and PR-1 proteins, have direct antimicrobial activity or are closely related to classes of antimicrobial proteins. The set of SAR genes that are induced in a plant may vary with the plant species. Although systemic acquired resistance does not affect spore germination and appressorium formation, penetration is reduced drastically in systemically induced resistant tissue, probably as a result of formation beneath the appressoria of papilla-like material that becomes impregnated quickly with lignin and silicon. In some host–pathogen systems, systemic acquired resistance is characterized by the induction of peroxidase and lipoxygenase activities that lead to the production of fatty acid derivatives, which exhibit strong antimicrobial activity. In plants exhibiting systemic acquired resistance in response to plant defense activators such as salicylic acid, bacterial growth and multiplication are reduced drastically (Fig. 6-25), although salicylic acid is tolerated by the bacteria at concentrations much higher than those found in the treated plant.

The mechanism of signal transduction in triggering systemic acquired resistance is still being studied. Salicylic acid seems to be involved in both the hypersensitive response and the systemic acquired resistance but may not be the signal that induces systemic acquired resistance (Fig. 6-26). Salicylic acid is present in the phloem of plants after the primary inoculation but before the onset of acquired resistance; its concentration levels correlate with the induction of PR proteins. External application of salicylic acid activates the same sets of SAR genes that are expressed after SAR induction by pathogens. Nevertheless, other evidence suggests that a signal other than salicylic acid is responsible for the systemic expression of systemic acquired resistance, but salicylic acid must be present for the real signal to be

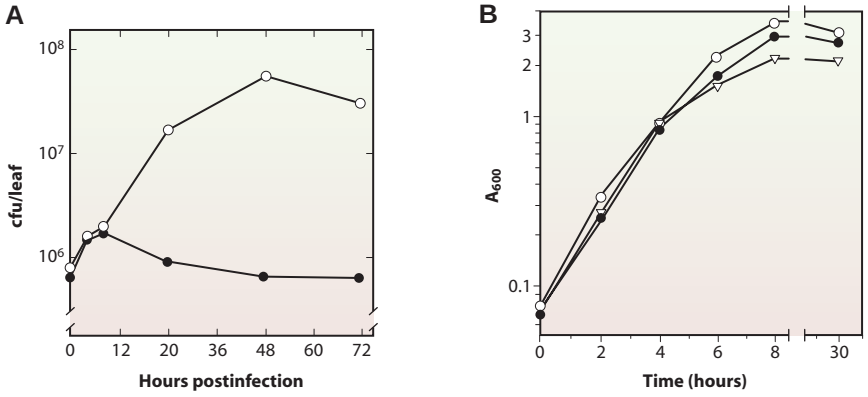


FIGURE 6-25 (A) Inhibition of growth and multiplication of *Erwinia carotovora* bacteria in inoculated leaves of tobacco seedlings growing in a medium containing 1 m*M* salicylic acid (●) or without salicyclic acid (○). cfu, colony-forming units (bacteria). Control leaves were nearly macerated 12 hours after inoculation, whereas salicylic acid-treated leaves had one small local lesion at the point of inoculation. (B) Lack of inhibition of growth and multiplication of the same bacteria in culture by various concentrations (0, 1, and 5 mM) of salicylic acid, indicating that the effect in A is caused by the plant defenses activated by salicylic acid and not by the salicylic acid itself. [From Palva *et al.* (1994) *Mol. Plant-Microbe Interact.* 7, 356–363.]

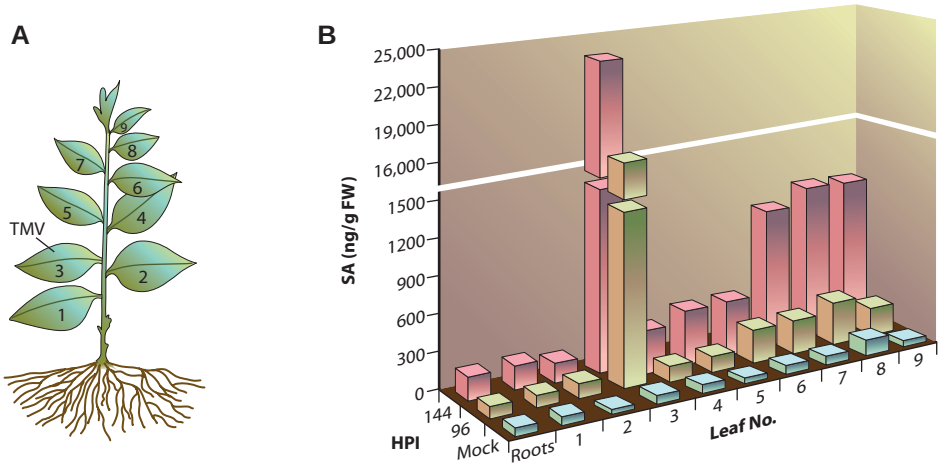


FIGURE 6-26 Salicylic acid accumulation throughout a 6-week-old tobacco plant after inoculation of a single leaf with a strain of tobacco mosaic virus that causes local lesions only and no systemic infection. (A) Inoculated leaf 3 in relation to other leaves and roots of the plant. (B) Concentrations of total salicyclic acid (SA, in nanograms per gram fresh weight) in the inoculated leaf (leaf 3) and in uninoculated roots and leaves at 96 and 144 hours postinoculation (HPI). MOCK, inoculation without virus. [From Shulaev *et al.* (1995) *Plant Cell* **7**, 1691–1701.]

transduced into gene expression and acquired resistance. It had been reported earlier that salicylic acid reacts with an oxidative enzyme (catalase) and generates reactive oxygen radicals. This had been suggested as a mechanism by which the plant cell reacts to salicylic acid signaling and induces systemic acquired resistance (Fig. 6-27). This notion, however, is no longer accepted.

The onset of systemic acquired resistance in Arabidopsis is controlled by a single gene, NPR1, which also affects local acquired resistance, i.e., the ability of plants to restrict the spread of virulent pathogen infections. Disruption of the gene produces mutant plants that fail to respond to a variety of SAR-inducing treatments, they display minimum expression of pathogenesis-related genes, and they exhibit increased susceptibility to infections by allowing lesions to grow and spread much more than in nonmutant plants. The NPR1 gene encodes a novel protein that contains ankyrin repeats and these repeats are needed for NPR1 to function. Also, when the NPR1 gene was inserted into a mutant that had lost the NPR1 gene, the mutant not only reacquired the responsiveness to SAR induction in terms of expression of PR genes and resistance to infection, the mutant transgenic plants actually became more resistant to infection by the bacterium *P. syringae* even in the absence of SAR induction. It was further shown that induction of NPR1 leads to overexpression of the NPR1-coded protein and this, in turn, induces the expression of numerous downstream pathogenesis-related genes. NPR1 seems to confer resistance to some bacterial and oomycete diseases in a dosage-dependent manner. The increased resistance provided by the overexpression of NPR1 seems to occur without any detrimental effects on the plants.

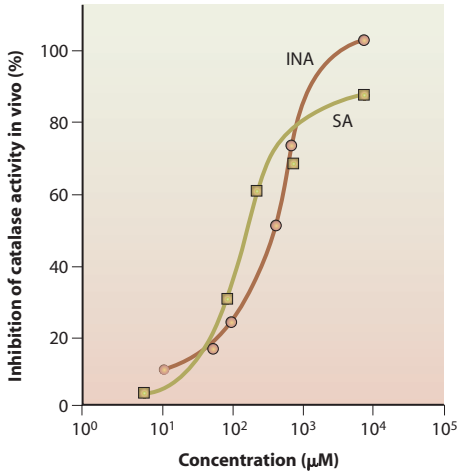


FIGURE 6-27 Inhibition of catalase activity by the plant defense-promoting compound salicyclic acid (SA) and the *in vivo*-produced active form of isonicotinic acid (INA). Such inhibition in resistant plants was earlier thought to result in the accumulation of active oxygen radicals and in the hypersensitive defense response. [From Conrath *et al.* (1995). *Proc. Natl. Acad. Sci. USA* **92**, 7143–7147.]

The induction of systemic acquired resistance through external application of salicylic acid raised the very important question of whether salicylic acid or other chemical compounds could be used to artificially induce systemic acquired resistance in plants against their numerous pathogens. Unfortunately, externally applied salicylic acid is not translocated efficiently in the plant and, in addition, salicylic acid is strongly

phytotoxic when applied at even slightly higher levels above the level required for efficacy. Therefore, salicylic acid per se has not been considered for use as a practical solution for disease control.

So far, in addition to salicyclic acid, derivatives of isonicotinic acid and benzothiazoles have been shown to induce systemic acquired resistance in plants against a variety of pathogens. As a matter of fact, the benzothiazole (BTH) is being used commercially. When the three compounds were used separately to protect barley against the powdery mildew fungus, they did so by inducing differential expression of a number of newly identified defense response genes, including genes encoding a lipoxygenase, a thionin, an acid phosphatase, a Ca^{2+}-binding protein, a serine proteinase inhibitor, a fatty acid desaturase, and several other proteins whose function had not been determined. Of the three chemicals, INA and BTH were more potent inducers of both gene expression and resistance. In experiments in which cowpea seeds were treated with BTH and were then inoculated with the anthracnose fungus *Colletotrichum destructivum*, the young cowpea plants were effectively protected from infection through a hypersensitive response of cells coming in contact with the pathogen. In addition, the plants showed a rapid transient increase of the phenoloxidizing enzymes phenylalanine ammonia lyase and chalcone isomerase while there was an early, accelerated accumulation of the phytoalexins kievitone and phaseollidin and of several other proteins. It was concluded that BTH protects cowpea seedlings by potentiating an early defense response rather than by altering the constitutive resistance of the tissues. The SAR-activating compounds induce expression of the same set of SAR genes that are induced either by salicylic acid or by various infectious agents and, in addition, seem to prime or sensitize plants to respond faster and with additional defense reactions than those characteristic of SAR genes. Isonicotinic acid, however, functions even in transgenic plants that are unable to accumulate salicylic acid. Apparently, therefore, isonicotinic acid triggers the signal transduction pathway that leads to SAR by acting either at the same site as salicylic acid or downstream from it.

Salicylic acid and isonicotinic acid are true SAR activators because not only do they induce resistance to the same spectrum of pathogens and induce expression of the same genes as pathogens, but these chemicals have no antimicrobial activity. Several other chemical compounds, such as the fungicides fosethyl-Al, metalaxyl, and triazoles, appear to have some resistance-inducing activity. The fungicide–bactericide probenazole is only slightly toxic *in vitro*, but induces various defense responses in rice plants, including an oxidative burst and appearance of reactive oxygen radicals, as well as significant accumulation of antimicrobial factors such as fungitoxic unsaturated fatty acids. A large number of other compounds, and also many microorganisms, have been tested for their ability to induce systemic acquired resistance in plants, but so far none has proved effective. This area of research, however, has a tremendous commercial potential, and therefore the search for SAR-inducing compounds is likely to continue and, actually, to increase.

DEFENSE THROUGH GENETICALLY ENGINEERING DISEASE-RESISTANT PLANTS

With Plant-Derived Genes

The number of plant genes for resistance (R genes) that have been isolated is increasing rapidly. The first plant gene for resistance to be isolated was the *Hml* gene of corn in 1992, which codes for an enzyme that inactivates the HC toxin produced by the leaf spot fungus *Cochliobolus carbonum*. In 1993, the Pto gene of tomato was isolated; this gene encodes a protein kinase involved in signal transduction and confers resistance to strains of the bacterium *P. syringae* pv. *tomato* that carry the avirulence gene *avrPto*. In 1994, four additional plant genes for resistance were isolated: the *Arabidopsis* RPS2 gene, which confers resistance to the strains of *P. syringae* pv. *tomato* and *P. syringae* pv. *maculicola* that carry the avirulence gene *avrRpt2*; the tobacco N gene, which confers resistance to tobacco mosaic virus; the tomato Cf9 gene, which confers resistance to the races of the fungus *Cladosporium fulvum* that carry the avirulence gene *avr9*; and the flax L^6 gene, which confers resistance to certain races of the rust fungus *Melampsora lini* carrying the avirulence gene *avr6*. The last five plant resistance genes are triggered into action by the corresponding avirulence genes of the pathogen, the products of which serve as signals that elicit the hypersensitive response in the host plant. Several more plant resistance genes have since been isolated. Some of these genes appear to provide plant resistance to pathogens expressing one or the other of two unrelated *avr* genes of the pathogen. It is expected that these and many other R genes, which are likely to be isolated in the years to come, will be used extensively in genetically engineering transgenic plants that will be resistant to many of the races of the pathogens that affect these plants.

In addition to these specific plant genes, several other plant genes encoding enzymes or other proteins (PR proteins) found widely among plants have been shown to confer resistance to transgenic plants in which they are expressed. For example, tobacco plants transformed with a chitinase gene from bean became resistant to

infection by the soilborne fungus *Rhizoctonia solani* but not to infection by the oomycete *Pythium aphanidermatum*, the cell walls of which lack chitin. In other experiments, constitutive expression of a PR chitinase gene from rice in transgenic rice and cucumber plants made the rice plants more resistant to *R. solani* and the cucumber plants more resistant to *Botrytis cinerea*. Similarly, transgenic tobacco plants expressing a PR1 protein gene were resistant to the blue mold oomycete *Peronospora tabacina*, and plants expressing the systemic acquired resistance gene SAR8.2 were resistant to the black shank oomycete *Phytophthora parasitica*. Also, transgenic soybean plants expressing a wheat gene for oxalate oxidase, which oxidizes oxalic acid, a pathogenicity factor for the soybean stem rot fungus *Sclerotinia sclerotiorum*, confers resistance to soybean by exhibiting its highest activity of oxalate oxidation in cell walls proximal to the site of pathogen attack. Moreover, transgenic potato plants expressing the gene for the antibacterial enzyme T4 lysozyme exhibited resistance to the soft rot and black leg caused by the bacterium *Erwinia carotovora* pv. *atroseptica*. Also, transgenic tobacco and potato plants expressing a gene from pokeweed (*Phytolacca* sp.) that codes for an antiviral, ribosome-inactivating protein exhibited resistance against several potato and other viruses. Plants are also aided in their defense from pathogens by plant-produced, ribosome-inactivating proteins (RIPs) that inhibit foreign protein synthesis in the cell without interfering with their own ribosomes. RIP genes also show synergism with PR protein genes when the two are expressed concurrently in the same plant.

Because mixtures of pathogenicity-related proteins are more effective as antimicrobials than each of them tested separately, it was soon shown that transgenic plants (tobacco) expressing both the chitinase and the β-1,3-glucanase were significantly more resistant to the fungi *Cercospora nicotianae* and *R. solani*, as was tomato to *Fusarium oxysporum* f. sp. *lycopersici*, than plants expressing either of the genes alone. Equally effective in providing plant resistance to fungi were hydrolytic enzymes, such as chitinase and glucanase, obtained organisms other than plants, such as the soilborne bacterium *Seratia marcescens*, or the human enzyme lysozyme. Other PR proteins, such as the defensins, a group of cysteine-rich, defense-related antimicrobial peptides constitutively present in the plasma membrane of most plant species, provide enhanced resistance to different pathogens.

Modification of existing plant genes that govern the external or internal cell surface receptor to which the virus binds may result in an inability of the virus to bind and to replicate in the cell and may lead to resistance or immunity. To these must also be added the induction of resistance in potato and tobacco transgenic plants transformed, respectively, with a mouse gene coding for an enzyme involved in the synthesis of an interferon-like compound and with a mouse gene coding for an antibody (plantibody) against the coat protein of a plant virus (*artichoke mottle crinkle virus*).

Additional mechanisms of enhancing the resistance of a plant with plant-derived genes include genetic engineering of plants with R genes that provide appropriate plant resistance or an elicitor molecule that triggers it; engineering plants with genes that overexpress one or more genes that regulate the systemic acquired resistance of the plant so that it (SAR) can be kept high continually and against a variety of pathogens; and by changing a previously compatible defense reaction to an incompatible (resistant) one through insertion of a resistance gene. Engineering plants with constitutive genes that trigger or enhance the accumulation of pathogenesis-related (PR) proteins, with genes such as stilbene synthase. This enzyme triggers the production of certain phytoalexins that subsequently reduce infection, e.g., of tobacco by *Botrytis cinerea*, by 50%. Or engineering plants with defective or less active genes that reduce the level of activity of calmodulin and of catalase, thereby leading to the production of continuously high levels of active oxygen species (H_2O_2), as well as the activated expression of PR proteins. Other types of plant genes engineered into plants for disease resistance include the lectin genes, which prevent plant infection by nematodes, and defensin genes that deter plant attacks by fungi. The use of known resistance genes, e.g., of Pto, Cf-9, and N, that protect certain tomato varieties from a bacterial spot, tomato from fungal black mold, and tobacco from mosaic virus, respectively, to confer resistance to different plants has, generally, not been successful. It appears that when a gene that confers strong resistance in one host is isolated and transferred to a different plant separated from its original genetic background, it is not able to confer resistance to the new plant.

With Pathogen-Derived Genes

In 1986, it was shown for the first time that tobacco plants transformed (genetically engineered) to express the coat protein gene of TMV showed various degrees of resistance to subsequent inoculation with the same virus. Once the TMV coat protein gene was integrated in the tobacco genome, it was carried through the seed and behaved like any other tobacco gene. Since then, numerous other crop plants, especially solanaceous ones such as tobacco, tomato, and pepper; legumes such as alfalfa; grains such as barley, corn, oats, and rice; cucurbits such as cucumber, cantaloupe, and squash; and

several other plants (papaya, impatiens, etc.), have been transformed with the coat protein gene of one or more of the viruses that infect them. The viruses from which the coat protein genes were obtained represent most of the virus groups.

In the vast majority of cases, transgenic plants show quite high levels of resistance to the virus from which the coat protein gene was derived and, in many cases, to other more or less related viruses. In some cases the transgenic plants were resistant to the virus if they were inoculated mechanically but not if inoculated by the specific vector of the virus, whereas in others the plants remained resistant even when inoculated by their aphid or fungus vector. In some cases, plants were transformed concurrently with as many as three viruses, the coat protein genes of which had been introduced in tandem into one location of the plant genome; such transgenic plants exhibited resistance to all three viruses.

Transgenic plants transformed with viral genes other than the coat protein gene often exhibit even higher levels of resistance to the virus providing the gene(s) and to, perhaps, additional viruses. Quite often the transferred genes either are portions of genes or are mutated artificially and thereby inactivated genes, so that they can be reproduced and expressed by the plant but do not produce a functional gene product that might aid a virus on infection. For example, highly resistant transgenic tobacco plants have been produced by transformation with modified virus replicase-coding genes of several viruses. Also, tobacco plants transformed with the TMV gene coding for the movement protein or for a dysfunctional movement protein are resistant to TMV and to several other viruses. Resistance to viruses has also been induced in plants transformed with viral genes coding for proteases needed for processing the viral nucleic acid, in plants transformed with small defective or satellite nucleic acids, and even in plants transformed with untranslatable or antisense segments of the viral nucleic acid.

Resistance to nonviral pathogens has also been increased through the engineering of plants with appropriate genes from pathogenic or nonpathogenic fungi and also from insects and other animals. For example, potato plants engineered to express the H_2O_2-generating glucose oxidase gene from the fungus *Aspergillus niger* continually produce high levels of peroxide ions in the apoplast of the plant cells, thereby increasing the resistance of the potato plants to the oomycete causing late blight (*Phytophthora infestans*), and the fungi causing early blight (*Alternaria solani*), and Verticillium wilt (*Verticillium dahliae*). The resistance of potato plants to the bacterial soft rot disease (caused by *Erwinia carotovora*), of tobacco plants to several fungal and bacterial diseases, and of apple plants to fire blight disease (caused by the bacterium *E. amylovora*) was increased when the plants were transformed with a hen, human, or T4 bacteriophage gene for lysozyme, which hydrolyzes the pteridoglycan layer of the bacterial cell wall and inhibits fungal and bacterial growth. Similarly, potato and apple plants transformed with the chitinase gene obtained from the fungus *Trichoderma harzianum,* which is used as a biocontrol agent against many plant pathogenic fungi, the walls of which it hydrolyzes with its chitinases, showed resistance to the potato early blight and to potato gray mold (caused by *Botrytis cinerea*), whereas the apple trees showed increased resistance to the apple scab disease (caused by the fungus *Venturia inaequalis*). Furthermore, tobacco, potato, apple, and pear plants showed increased resistance when transformed with certain genes; some genes were obtained from insects and code for antibacterial proteins, such as cecropins, which are lytic peptides that make pores in and cause lysis of bacterial cell membrane; or transformed with the genes coding for the antimicrobial proteins known as attacins, which inhibit the synthesis of the outer membrane protein in gram-negative bacteria. Such genes increased resistance bacterial wildfire of tobacco (caused by *P. syringae* pv. *tabaci*), of potato to bacterial black leg (caused by *E. carotovora* subsp. *atroceptica*), and of apple and pear to fire blight (caused by *E. amylovora*).

There is every expectation that the area of inducing plant resistance to pathogens through genetic transformation with pathogen-derived genes will grow and improve rapidly. Such genetic engineering strategies will provide an excellent additional tool for plant disease control.

DEFENSE THROUGH RNA SILENCING BY PATHOGEN-DERIVED GENES

RNA silencing is a type of gene regulation that, in plants, serves as an antiviral defense. RNA silencing is based on targeting specific sequences of RNA and degrading them. RNA silencing occurs in a broad range of eukaryotic organisms, including plants, fungi, and animals. While plants use RNA silencing to defend themselves against viruses, the viruses, in turn, encode proteins by which they attempt to suppress the silencing of their RNA. The consensus is that RNA silencing is one of the many interconnected pathways for RNA surveillance and cell defense.

RNA silencing was first observed in transgenic plants transformed with viral genes providing "pathogen-derived resistance." It was noticed then that sense orientation genes in the transgenic plant interfered with the expression of both the transgenes themselves and related

endogenous genes of the plant. Because of the concurrent suppression of both genes, RNA silencing was at first called "cosuppression." RNA silencing is due to a process that occurs after transcription (posttranscriptional gene silencing) of the RNA and involves targeted mRNA degradation. Clues of its existence came from the discovery that plants carrying viral transgenes were resistant to related strains of the virus that replicate in the cytoplasm, which meant that silencing occurs in the cytoplasm rather than the nucleus. The nucleotide sequence specificity of the RNA depends on the sequence of 21–25 nucleotides of antisense RNA produced directly or indirectly from sense transgenes, or from dsRNA. The dsRNA is a trigger or an intermediate in the cleaving into small (21–25 nucleotides), sense or antisense RNAs called small interfering (siRNAs). siRNAs act as guides that direct the RNA degradation machinery [the RNA-induced silencing complex (RISC)] to the target RNAs.

The main events in RNA silencing, as understood at this point in time, include the following steps (Fig. 6-28): A plant or viral gene is inserted in the plant DNA where it is expressed and produces messenger RNA (mRNA). The viral gene may also be able to do that without being inserted in the plant genome. RNA viruses routinely produce double-stranded RNA (dsRNA), and RNA from some abnormal genes doubles up upon itself and forms "aberrant" dsRNA. Both dsRNAs are cleaved by an enzyme called "Dicer" into small interfering RNAs about 21–25 nucleotides long. The siRNA fragments split into individual ssRNAs and these combine with proteins and produce an RNA-induced silencing complex (RISC). This complex captures mRNAs that complement each short RNA sequence. RNAs with a nearly perfect match of their sequence with that of small RNA are sliced into useless small fragments. RNAs with less perfect sequence matches cause the RISC complex to block the movement of the ribosomes on the mRNA so that the mRNA is not translated and does not produce a protein, thereby silencing that RNA.

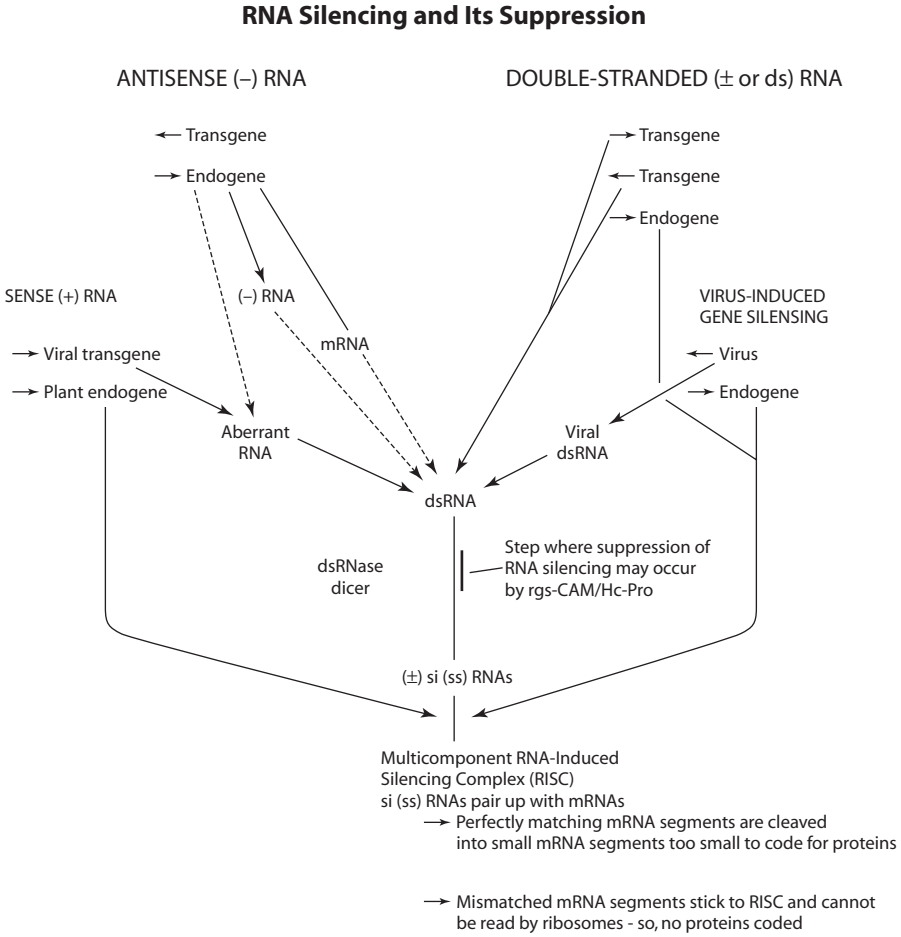


FIGURE 6-28 Diagram of the steps, some of them hypothetical, involved in the in-cell silencing of transgene, endogene, and viral RNA as a mechanism of plant defense. [Modified from Vance and Vaucheret (2001). *Science* **222**, 2277–2280.]

RNA silencing produces exceptionally strong virus resistance in transgenic plants. Such plants have neither detectable accumulation of virus in their inoculated leaves nor can this resistance be overcome with high-titer inocula. Once RNA silencing of the transgene is established, all RNAs homologous to the transgene, including those from an infecting virus, are degraded. Also, although RNA silencing is triggered locally, it can spread throughout the plant via a mobile silencing signal. The movement of the silencing signal in the plant parallels that of the virus, moving at first from cell to cell and then entering the phloem and from there spreading out to parenchyma cells again. The parallel movement of the virus and RNA-silencing signal may represent a race between the two, with the out-come of the race being a successful infection if the virus moves faster and becomes established first, or resistance, i.e., lack of infection, if RNA silencing becomes established first.

It was later shown that plant viruses could also induce RNA silencing. It was further shown that virus-induced gene silencing (VIGS) could be directed to either transgenes in the plant or endogenous genes of the plant. As a result, plant viruses could both induce RNA silencing and could be targeted for RNA silencing by transgenes. VIGS, however, is rather mild, transient, and restricted to regions around the veins. RNA silencing has not yet been reported to occur in plant DNA viruses, both the ssDNA geminiviruses and the reverse-transcribing dsDNA viruses. All DNA viruses, however, seem to have the potential to induce gene silencing in the nucleus and in the cytoplasm, as they produce multiple copies of viral DNA genomes in the nucleus, show illegitimate integration of viral DNA into host chromosomes that mimics transgene transformation for such viruses, and generate a great deal of viral RNAs in the cytoplasm.

Suppressors of RNA Silencing

Soon after the discovery of RNA silencing, it was discovered that many plant viruses encode proteins that suppress RNA silencing. The suppressors are structurally diverse and seem to have undergone repeated evolution steps in their attempt to keep up with developments in RNA silencing. One suppressor, the helper component-proteinase of potyviruses, is so effective in suppressing viral RNA silencing that it actually increases the accumulation of several unrelated plant viruses and is, possibly, responsible for the many potyvirus-associated synergistic diseases of plants. The same suppressor prevents both virus-induced and transgene-induced RNA silencing and can even reverse an already established RNA silencing of a transgene. The suppression induced by the potyvirus suppressor to a transgene-induced RNA silencing can be reversed at a step at which the accumulation of siRNAs is eliminated, but it cannot eliminate the mobile silencing signal. Another suppressor, *the potato virus X* p25 protein, is much less effective in suppressing RNA silencing and it apparently targets and interferes with systemic silencing.

In addition to the suppression of RNA silencing by virus-encoded proteins, RNA silencing can also be suppressed by certain host genes. Some of these genes are expressed in transgenic plants, in plants following infection with certain viruses, and in transgenic plants carrying the potyvirus suppressor protein. These observations suggest that the host–coded suppressor acts as a relay for the potyvirus suppressor-mediated suppression of post-transcriptional gene silencing or that the potyvirus suppressor-induced suppression of silencing perhaps takes place via activation of the host-induced suppressor protein and its unknown target protein.

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chapter seven

ENVIRONMENTAL EFFECTS *ON THE* DEVELOPMENT *OF* INFECTIOUS PLANT DISEASE

Plant diseases occur in all parts of the world where plants grow. They are more common and more severe, however, in humid to wet areas with cool, warm, or tropical temperatures (Fig. 7-1). Plants in dry areas may not be subjected to as many severe fungal, bacterial, or nematode diseases, but they are often attacked severely by powdery mildew fungi, by xylem-inhabiting fastidious bacteria, by phloem-inhabiting phytoplasmas and fastidious bacteria, and by viruses transmitted by certain insect vectors.

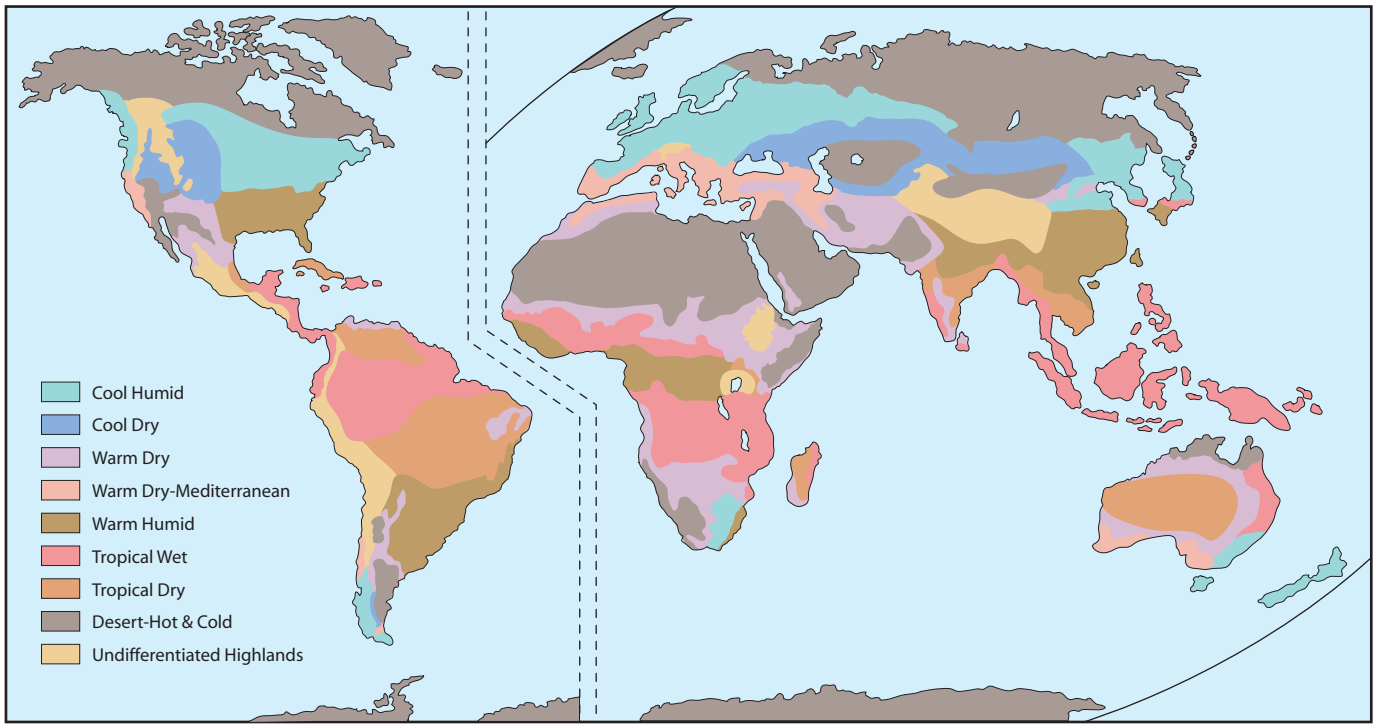

FIGURE 7-1 World map of agricultural climates. [From Duckham and Masefield (1970), "Farming Systems of the World." Chatto and Windus, London.]

Although all pathogens, all perennial plants, and, in warmer climates, many annual plants are present in the field throughout the year, almost all diseases, in all but a few very hot, dry areas, occur only, or develop best, during the warmer part of the year. Also, it is common knowledge that most diseases appear and develop best during wet, warm days and nights and that plants fertilized heavily with nitrogen are attacked much more severely by some pathogens than less fertilized plants. These general examples clearly indicate that the environmental conditions prevailing in both air and soil, after contact of a pathogen with its host, may affect the development of the disease greatly. Actually, environmental conditions frequently determine whether a disease will occur. The environmental factors that affect the initiation and development of infectious plant diseases most seriously are temperature and moisture on the plant surface (Fig. 7-2). Soil nutrients also play an important role in some diseases and, to a lesser extent, light and soil pH. These factors affect disease development through their influence on the growth and susceptibility of the host, on the multiplication and activity of the pathogen, or on the interaction of host and pathogen as it relates to the severity of symptom development.

As mentioned previously, for a disease to occur and to develop optimally, a combination of three factors must be present: susceptible plant, infective pathogen, and favorable environment. However, although plant susceptibility and pathogen infectivity remain essentially unchanged in the same plant for at least several days, and sometimes for weeks or months, the environmental conditions may change more or less suddenly and to

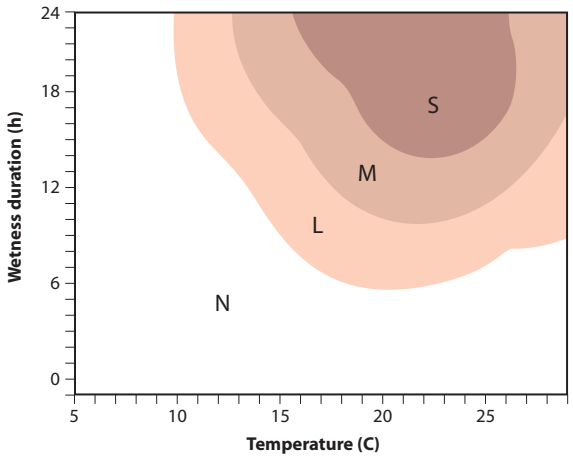

FIGURE 7-2 Various combinations of temperature and wetness duration at the fruit surface result in different severity levels of brown spot disease of pears, caused by the fungus *Stemphylium vesicarium*. N, none; L, light (20–40%); M, moderate (40–70%); S, severe (>70%). [From Montesinos *et al.* (1995). *Phytopathology* 85, 586–592.]

various degrees. Such changes may drastically influence the development of diseases in progress or the initiation of new diseases. Of course, a change in any environmental factor may favor the host, the pathogen, or both or it may be more favorable to one than it is to the other. As a result, the expression of disease will be affected accordingly. Plant diseases generally occur over a fairly wide range of the various environmental conditions. Nevertheless, the extent and frequency of disease occurrence, as well as the severity of the disease on individual plants, are influenced by the degree of deviation of each environmental condition from the point at which disease development is optimal.

EFFECT OF TEMPERATURE

Plants, as well as pathogens, require certain minimum temperatures to grow and carry out their activities (Fig. 7-1). In temperate regions, the low temperatures of late fall, winter, and early spring are below the minimum required by most pathogens. Therefore, diseases are not, as a rule, initiated during that time, and those in progress generally come to a halt. With the advent of higher temperatures, however, pathogens become active and, when other conditions are favorable, they can infect plants and cause disease. For example, in many canker diseases of perennial plants caused by fungi such as *Nectria*, *Leucostoma* (*Cytospora*), the oomycete *Phytophthora* or by bacteria such as *Pseudomonas*, infections begin and develop primarily in early spring or in the fall. The reason is that during these periods the temperatures are high enough for these fungi to grow well (Fig. 7-3) but are too low to allow optimum host development. Development of the same diseases stops during the winter when temperatures are too low for both host and pathogen, and it is quite reduced during the summer months when host growth and host defenses are at their optimum.

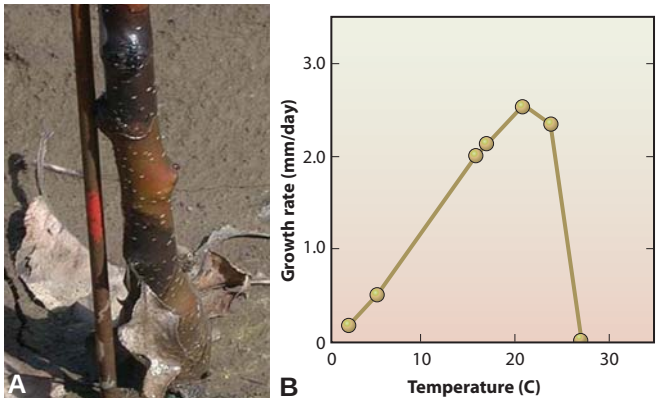

FIGURE 7-3 (A) Two cankers on a stem of a young pear tree caused by the oomycete *Phytophthora*. (B) Effect of temperature on the rate of growth of the canker-causing oomycete *Phytophthora syringae*. [Courtesy of (A) R. Regan, Oregon State University and (B) Bostock and Doster (1985). *Plant Dis*. **69**, 568–571.]

Pathogens differ in their preference for higher or lower temperatures. Some fungi grow much faster at lower temperatures (Fig. 7-3) than others (Fig. 7-4), and there may be significant differences among races of the same fungus (Fig. 7-4). Temperature affects the number of spores formed in a unit plant area (Figs. 7-5A and 7-5B) and the number of spores released in a given time period (Figs. 7-5A, 7-5C, and 7-9). As a result, many diseases develop best in areas, seasons, or years with cooler temperatures, whereas others develop best where and when relatively high temperatures prevail. Thus, some species of the fungi *Typhula* and *Fusarium*, which cause snow mold of cereals and turf grasses, thrive only in cool seasons or cold regions. Also, the late blight pathogen *Phytophthora infestans* is most serious in the northern latitudes; in the subtropics it is serious only during the winter. Many diseases, such as the brown rot of stone fruits caused by *Monilinia fructicola*, are favored by relatively high temperatures and are limited in range to areas and seasons in which such temperatures are prevalent. Several diseases, such as the fusarial wilts, many anthracnoses caused by *Colletotrichum*, and the bacterial wilts of solanaceous plants caused by *Ralstonia solanacearum*, are favored by high temperatures and are limited to hot areas, being particularly severe in the subtropics and tropics.

The effect of temperature on the development of a particular disease after infection depends on the specific host–pathogen combination. The most rapid disease development, i.e., the shortest time required for the completion of an infection cycle, usually occurs when the temperature is optimum for the development of the pathogen but is above or below the optimum for the development of the host. At temperatures much below or above the optimum for the pathogen, or near the optimum for the host, disease development is slower. Thus, for stem rust of wheat, caused by *Puccinia graminis tritici*, the time required for an infection cycle (from inoculation with uredospores to the formation of new uredospores) is 22 days at 5°C, 15 days at 10°C, and 5 to 6 days at 23°C. Similar time periods for the completion of an infection cycle are required in many other diseases caused by fungi, bacteria, and nematodes. Because the duration of an infection cycle determines the number of infection cycles and, therefore, the number of new infections in one season, it is clear that the effect of temperature on the prevalence of a disease in a given season may be very great.

If the minimum, optimum, and maximum temperatures for the pathogen, the host, and the disease are

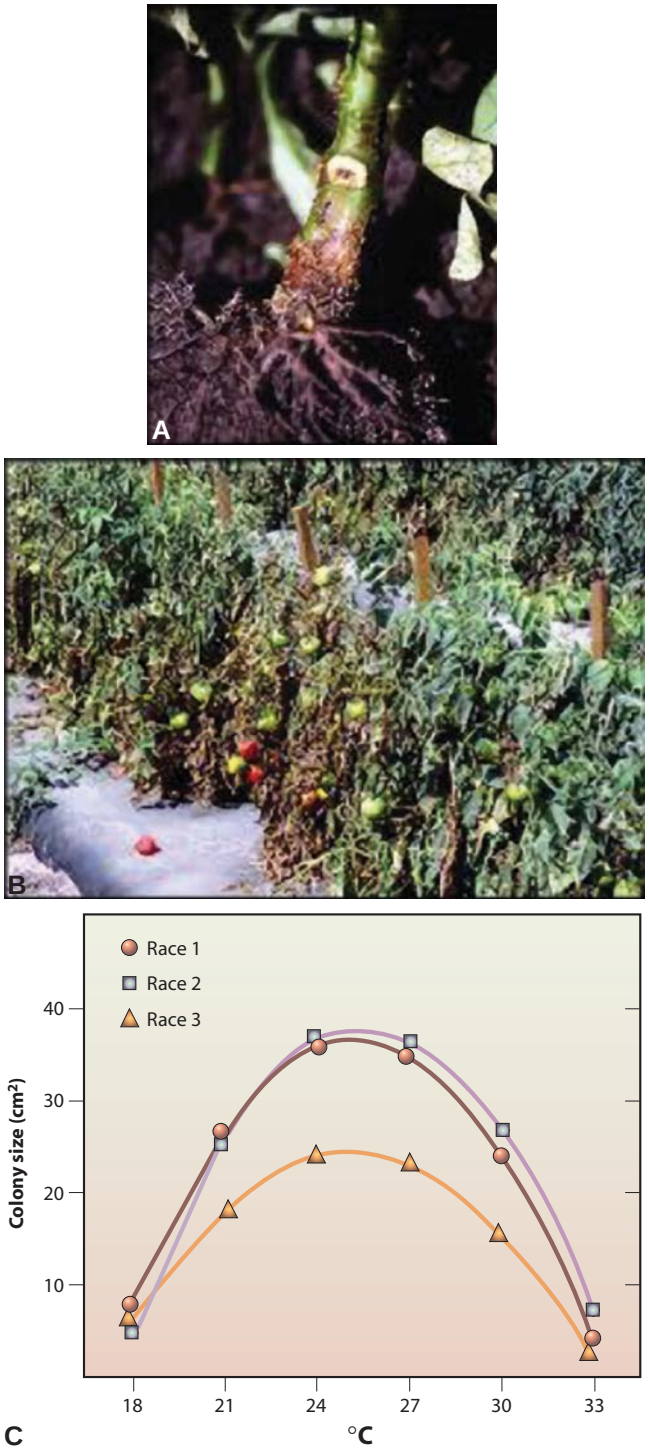

FIGURE 7-4 (A) Root and crown rot of tomato plant caused by *Fusarium oxysporum*. (B) Aboveground symptoms of wilting and death of tomato plants affected by such root and crown rot. (C) Effect of temperature on growth of *F. oxysporum* and difference in the growth of some of its races at the same temperatures. [Courtesy of (A and B) R. J. McGovern, University of Florida and (C) Swanson and van Gundy (1985). *Plant Dis.* **69**, 779–781.]

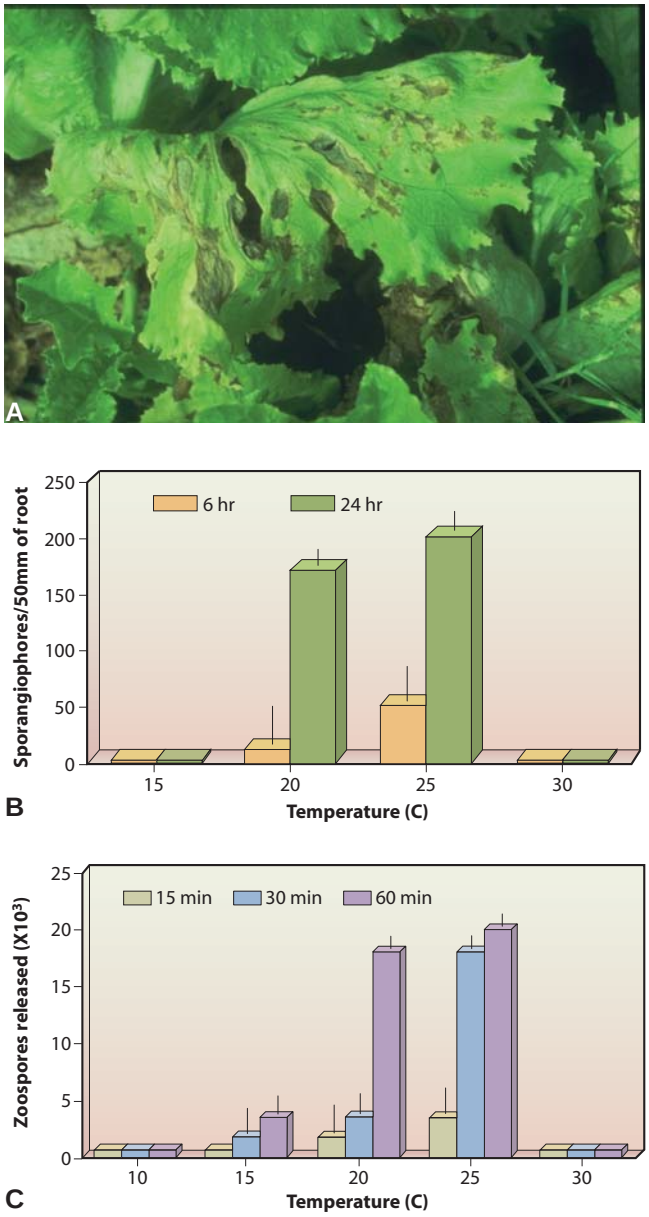

FIGURE 7-5 (A) Lettuce leaves showing symptoms of downy mildew caused by *Bremia lactucae*. (B) Effect of 6- and 24-hour temperature exposures on the production of sporangiophores (top) on infected roots and of 15-, 30-, and 60-minute temperature exposures on zoospores released from sporangia (bottom) of the same oomycete. [Courtesy of University Florida and (B) Stanghellini *et al.* (1990). *Plant Dis.* **74**, 173–178.]

about the same, the effect of temperature in disease development is apparently through its influence on the pathogen. The latter becomes so activated at the optimum temperature that the host, even at its optimum growth rate, cannot contain it.

In many diseases, the optimum temperature for disease development seems to be different from those of both pathogen and host. Thus, in the black root rot of tobacco, caused by the fungus *Thielaviopsis basicola*, the optimum temperature range for disease is 17 to 23°C, that for tobacco growth is 28 to 29°C, and that for the pathogen is 22 to 28°C. Evidently, neither the pathogen nor the host grow well at 17 to 23°C, but the host grows so much more poorly and is so much weaker than the pathogen that even the weakened pathogen can cause maximum disease development. In the root rots of wheat and corn caused by the fungus *Gibberella zeae*, the maximum disease development on wheat occurs at temperatures above the optima for the development of both the pathogen and wheat, but on corn it occurs at temperatures below the optima for the pathogen and corn. Considering that wheat grows best at low temperatures whereas corn grows best at high temperatures, it would appear that the more severe damage to wheat at high temperatures and to corn at low temperatures is due to a disproportionately greater weakening of the plants than of the pathogen at the unfavorable temperatures.

The effect of temperature on virus diseases of plants is much more unpredictable. In virus inoculation experiments in the greenhouse, temperature determines not only the ease with which plants can become infected with a virus, but also whether a virus multiplies in the plant and, if it does, the type of symptoms produced. The severity of the disease may vary greatly in various virus–host combinations depending on the temperature during certain stages of the disease. In the field, temperature, probably in combination with sunlight, seems to determine the seasonal appearance of symptoms in the various virus diseases of plants. Viruses producing yellows or leaf-roll symptoms are most severe in the summer, whereas those causing mosaic or ring spot symptoms are most pronounced in the spring. New growth produced during the summer on mosaic- or ring spot-infected plants usually shows only mild symptoms or is completely free from symptoms.

It is now becoming clear that temperatures, high or low, operate by affecting the genetic machinery of the cell by favoring or inhibiting the expression of certain genes involved in disease resistance or susceptibility. For example, cold hardening increases the resistance of cereals and grasses to the snow mold disease caused by the fungus *Microdochium nivale,* partly by causing an increase in sucrose synthetase and, upon infection, in a more rapid production by the plant of pathogenesis-related proteins. However, exposure of barley leaves to 50°C for one minute resulted in induced resistance against the powdery mildew fungus *Blumeria graminis* f. sp. *hordei* by causing an oxidative burst in the plant, production of cell wall-bound proteins, and stoppage of fungal growth after appressorium formation.

EFFECT OF MOISTURE

Moisture, like temperature, influences the initiation and development of infectious plant diseases in many interrelated ways. It may exist as rain or irrigation water on the plant surface or around the roots, as relative humidity in the air, and as dew. Moisture is indispensable for the germination of fungal spores and penetration of the host by the germ tube. It is also indispensable for the activation of bacterial, fungal, and nematode pathogens before they can infect the plant. Moisture, in such forms as splashing rain and running water, also plays an important role in the distribution and spread of many of these pathogens on the same plant and on their spread from one plant to another. Finally, moisture increases the succulence of host plants and thus their susceptibility to certain pathogens, which affects the extent and severity of disease.

The occurrence of many diseases in a particular region is closely correlated with the amount and distribution of rainfall within the year (Fig. 7-1). Thus, late blight of potato, apple scab, downy mildew of grapes, and fire blight are found or are severe only in areas with high rainfall or high relative humidity during the growing season. Indeed, in all of these and other diseases, the rainfall determines not only the severity of the disease, but also whether the disease will even occur in a given season (Fig. 7-6). In fungal diseases, moisture affects fungal spore formation, longevity, and particularly the germination of spores, which requires a film of water covering the tissues. In many fungi, moisture also affects the liberation of spores from the sporophores, which, as in apple scab, can occur only in the presence of moisture. The number of infection cycles per season of many fungal diseases is closely correlated with the number of rainfalls per season, particularly of rainfalls that are of sufficient duration to allow establishment of new infections. Thus in apple scab, for example, continuous wetting of the leaves, fruit, and so on for at least 9 hours is required for any infection to take place even at the optimum range (18 to 23°C) of temperature for the pathogen. At lower or higher temperatures the minimum wetting period required is higher, e.g., 14 hours at 10°C and 28 hours at 6°C. Similar conditions are required for the initiation and development of

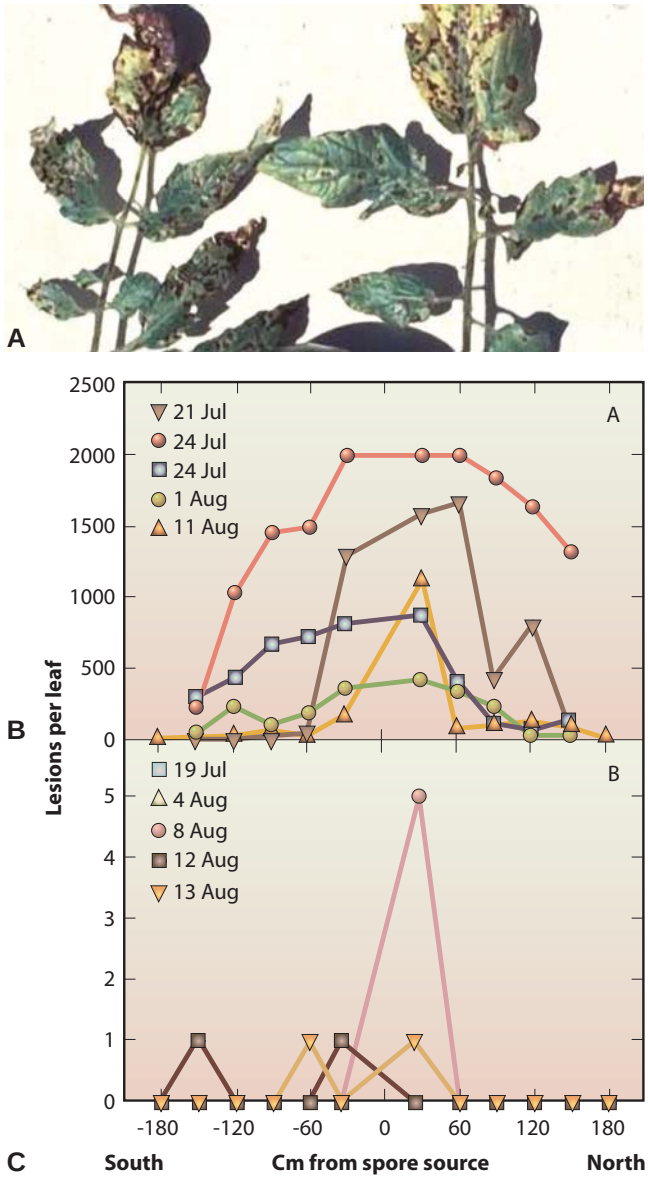

FIGURE 7-6 (A) Foliar symptoms of tomato infected by *Septoria lycopersici*. (B and C) Effect of moisture (rain) on the number of *Septoria* leaf spots per leaf on tomato plants planted at 30-centimeter intervals from an inoculated row (0) on five dates in the presence (A) and absence (B) of rainfall. The amounts of the five successive rainfalls in (A) on the dates indicated were 19.1, 30.0, 11.9, 4.1, and 8.6 millimeters [Courtesy of University of Florida and (B and C) Parker *et al*. (1995). *Plant Dis*. **79**, 148–152.]

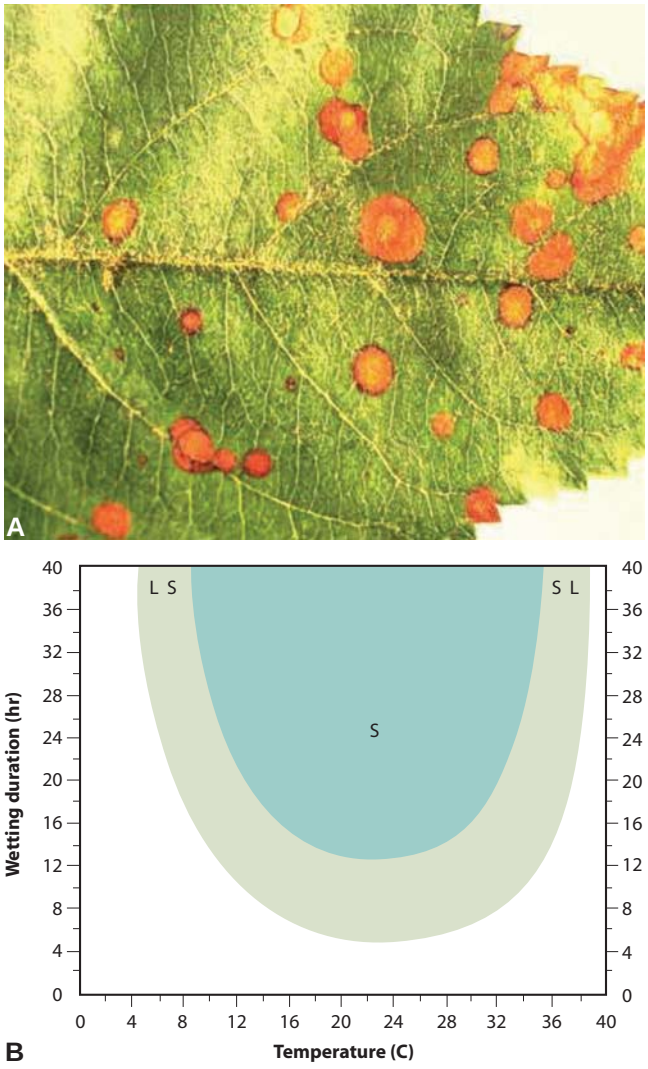

FIGURE 7-7 (A) Apple leaf spot caused by *Alternaria mali*. (B) Leaf wetness and temperature requirements for the leaf-spotting fungus *A. mali* to cause light (L) or severe (S) infection. (Photographs courtesy of T. B. Sutton, North Carolina State University.)

infections in many other diseases (Figs. 7-7 and 7-8). If the length of the wetting period is less than the minimum required for the particular temperature, the pathogen fails to establish itself in the host and fails to produce disease.

Most fungal pathogens require free moisture on the host or high relative humidity in the atmosphere for spore release (Fig. 7-9) or for germination of their spores. Most pathogens become independent of outside moisture once they can obtain nutrients and water from the host. Some pathogens, however, such as those causing late blight of potato and the downy mildews (Fig. 7-9A), must have high relative humidity or free moisture in the environment throughout their development. In these diseases, although spores may be released following a short leaf-wetness period (Figs. 7-9B and 7-9C), the growth and sporulation of the pathogen, and the production of symptoms, come to a halt as soon as dry, hot weather sets in. All these activities resume only when it rains again or after the return of humid weather.

Although most fungal and bacterial pathogens of aboveground parts of plants require a film of water to infect hosts successfully, spores of the powdery mildew

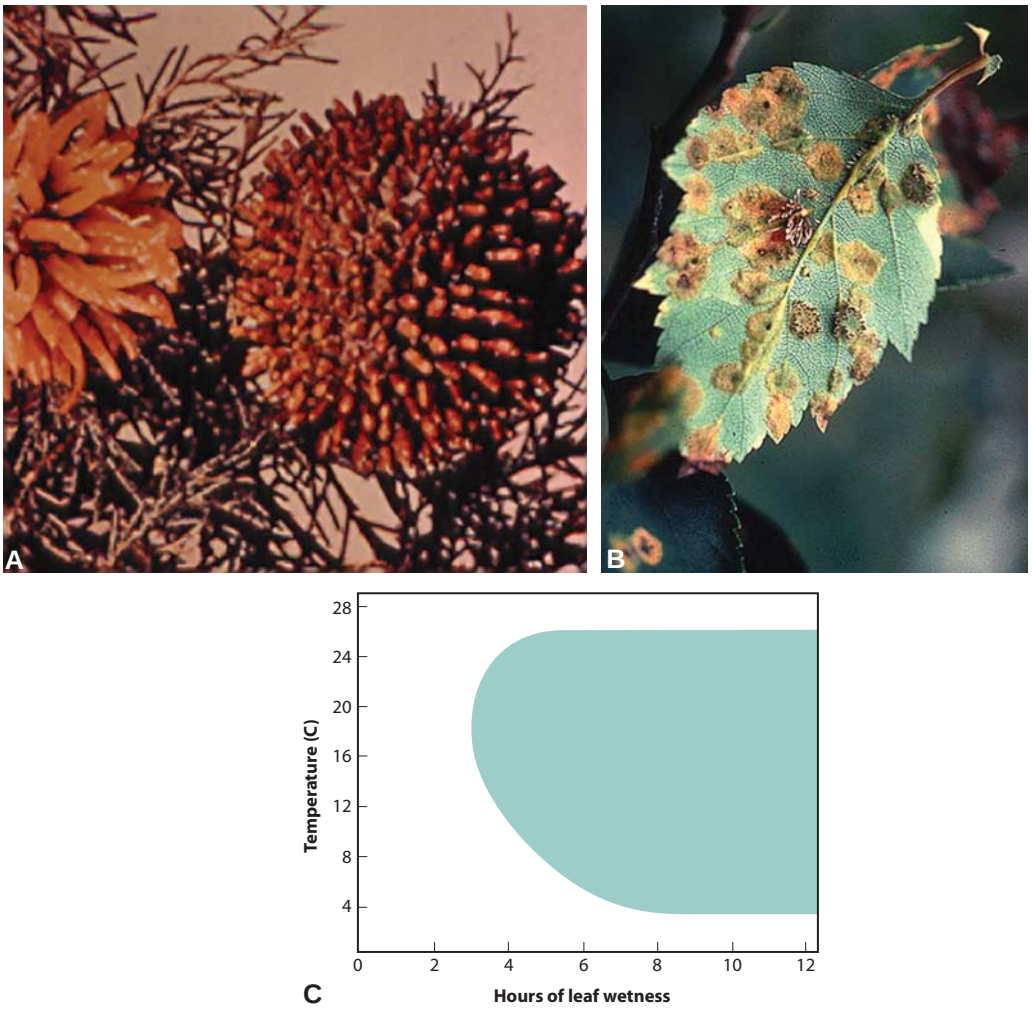

FIGURE 7-8 (A) Cedar-apple rust, caused by the fungus *Gymnosporangium juniperi-virginianae,* produces "cedar apples" on cedar. (B) Cedar-apple rust leaf spots on apple leaf resulting from basidiospores produced on cedar-apple telial horns. (C) Formation of basidiospores occurs when the temperature–leaf wetness point is at the transition line between the clear and shaded area of the diagram. If the temperature–leaf wetness point is within the shaded area, spore germination has occurred and infection is likely. [Photographs courtesy of University of Florida, (B) J. A. Christensen, Texas A&M University, and (C) Seem and Russo (1984). *Plant Dis.* **68**, 656–660.]

fungi can germinate, penetrate, and cause infection even when there is only high relative humidity in the atmosphere surrounding the plant. In powdery mildews, spore germination and infection are actually lower in the presence of free moisture on the plant surface than they are in its absence. In some of them, the most severe infections take place when the relative humidity is rather low (50 to 70%). In these diseases, the amount of disease is limited rather than increased by wet weather, as indicated by the fact that powdery mildews are more common and more severe in the drier areas of the world. The relative importance of powdery mildews decreases as rainfall increases. In high rainfall areas and periods, other diseases become more prevalent.

In many diseases affecting underground parts of plants, such as roots, tubers, and young seedlings, e.g., in the *Pythium* damping off of seedlings and seed decays, the severity of the disease is proportional to the amount of soil moisture and is greatest near the saturation point. The increased moisture seems to affect primarily the pathogen, which multiplies and moves (zoospores in the case of *Pythium*) best in wet soils. Increased moisture may also decrease the ability of the host to defend itself through a reduced availability of oxygen in water-logged soil and by lowering the temperature of such soils. Many other soil pathogens [e.g., *Phytophthora* (Fig. 7-10A), *Rhizoctonia*, *Sclerotinia*, and *Sclerotium*], some bacteria (e.g., *Erwinia* and

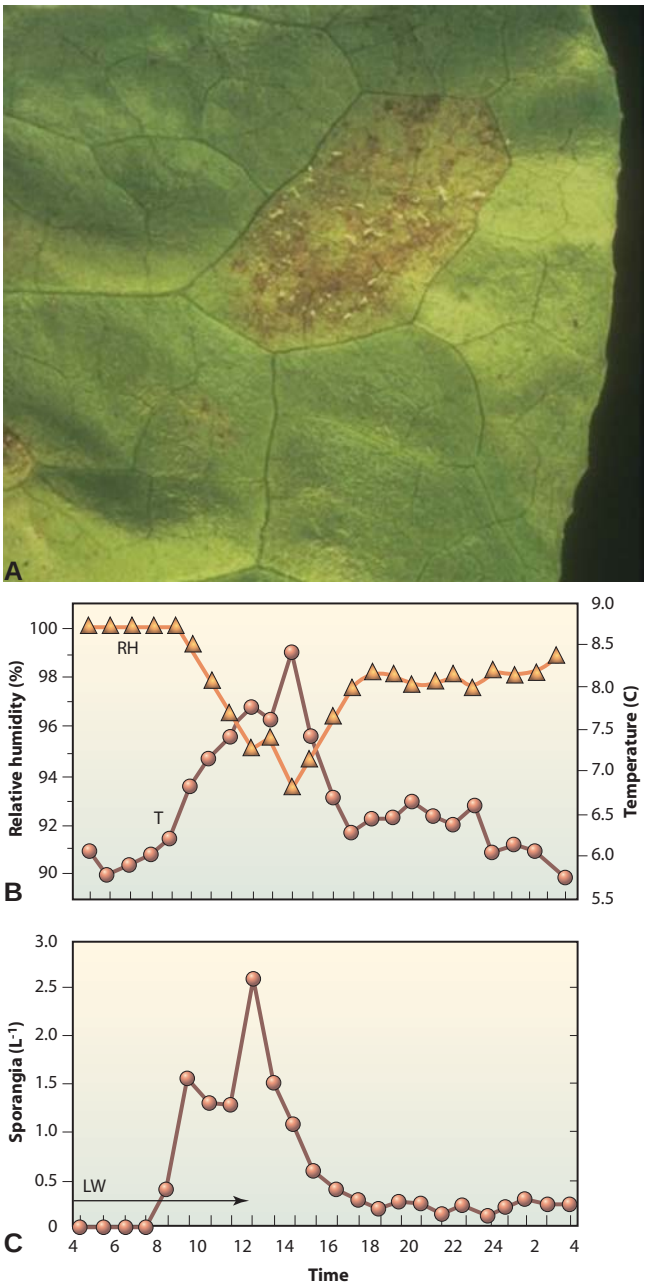

FIGURE 7-9 (A) A large and some smaller areas on a downy mildew–infected lettuce leaf producing sporangiophores and sporangia. (B) Relationship of temperature (T), relative humidity (RH), and spore release by *Bremia lactucae*, the cause of a downy mildew of lettuce, in a 24-hour period following a leaf wetness (LW) period (arrow). [Photographs courtesy of University of Florida and (B) Scherm and van Bruggen (1995). *Phytopathology* **85**, 552–555.]

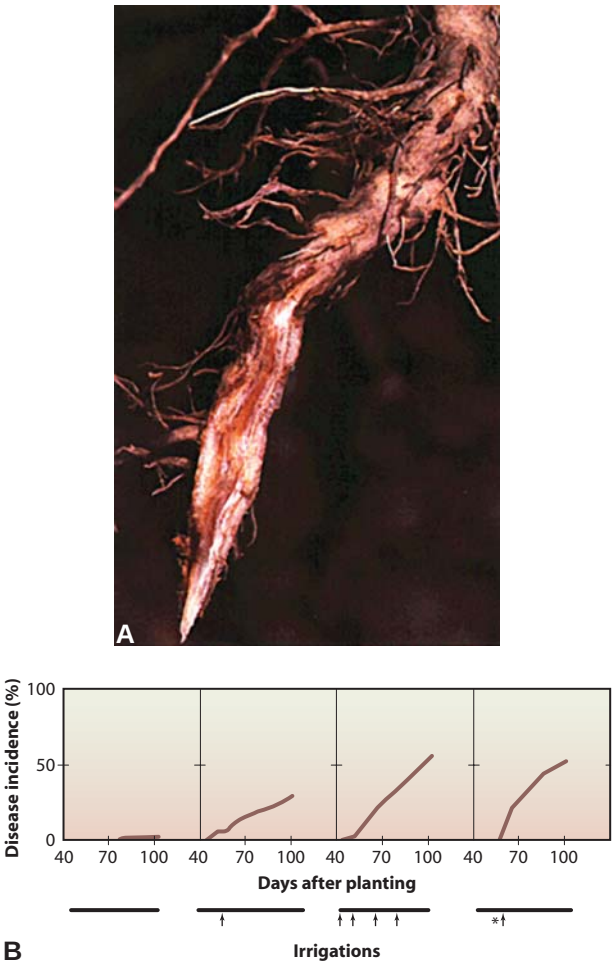

FIGURE 7-10 (A) Root rot symptoms caused by *Phytophthora sp. (B)* Development of *Phytophthora* root rot in a susceptible safflower variety under various irrigation schedules in the field. There was no rainfall. Arrows show times of surface irrigations. The asterisk before the arrow at the right field denotes plant stress before irrigation. (B) From Duniway (1982). *In* "Biometeorology in IPM" (Hatfield and Thomason, eds.). Academic Press, New York.

Pseudomonas), and most nematodes usually cause their most severe symptoms on plants when the soil is wet but not flooded (Fig. 7-10B). Several other fungi, e.g., *Fusarium solani*, which is the cause of dry root rot of beans, *Fusarium roseum*, the cause of seedling blights, and *Macrophomina phaseoli*, the cause of charcoal rot of sorghum and of root rot of cotton, grow fairly well in rather dry environments. Apparently that characteristic enables them to cause more severe diseases in drier soils on plants that are stressed by insufficient water. Vascular wilts caused by the fungus *Verticillium* and canker diseases of forest trees and seedlings caused by fungi are significantly more severe when the plants suffer from water stress. Similarly, *Streptomyces scabies*, which causes the common scab of potatoes, becomes most severe in soils drying out after wetting.

Most bacterial diseases, and also many fungal diseases of young tender tissues, are particularly favored by high moisture or high relative humidity. Bacterial pathogens and fungal spores are usually disseminated in water drops splashed by rain, in rainwater moving from the surfaces of infected tissues to those of healthy ones, or in free water in the soil. Bacteria penetrate plants through wounds or natural openings and cause severe

disease when present in large numbers. Once inside the plant tissues, the bacteria multiply faster and are more active during wet weather, probably because the plants, through increased water absorption and resulting succulence, can provide the high concentrations of water that favor bacteria. The increased bacterial activity in wet weather produces greater damage to tissues. This damage, in turn, helps release greater numbers of bacteria onto the plant surface, where they are available to start more infections if the wet weather continues.

EFFECT OF WIND

Wind influences infectious plant diseases primarily by increasing the spread of plant pathogens and the number of wounds on host plants and, to a smaller extent, by accelerating the drying of wet surfaces of plants. Most plant diseases that spread rapidly and are likely to assume large epidemic proportions are caused by pathogens such as fungi, bacteria, and viruses that are spread either directly by the wind or indirectly by insect vectors that can themselves be carried over long distances by the wind. Some spores, e.g., basidiospores, and some conidia, and also zoosporangia, are quite delicate and do not survive long-distance transport in the wind. Others, e.g., uredospores and many kinds of conidia, can be transported by the wind for many kilometers. Wind is even more important in disease development when it is accompanied by rain. Wind-blown rain helps release spores and bacteria from infected tissue and then carries them through the air and deposits them on wet surfaces of plants, which, if susceptible, can be infected immediately. Wind also injures plant surfaces while they are blown about and rub against one another or through wind-blown sand; this facilitates infection by many fungi and bacteria and also by a few mechanically transmitted viruses. Wind, however, sometimes helps prevent infection by accelerating the drying of the wet plant surfaces on which fungal spores or bacteria may have landed. If the plant surfaces dry before penetration has taken place, any germinating spores or bacteria present on the plant are likely to desiccate and die, and no infection will occur.

EFFECT OF LIGHT

The effect of light on disease development, especially under natural conditions, is far less than that of temperature or moisture. Several diseases are known in which the intensity and the duration of light may either increase or decrease the susceptibility of plants to infection and also the severity of the disease. In nature, however, the effect of light is limited to the production of more or less etiolated plants as a result of reduced light intensity. This usually increases the susceptibility of plants to nonobligate parasites, for example, of lettuce and tomato plants to *Botrytis* or of tomato to *Fusarium*, but decreases their susceptibility to obligate parasites, for example, of wheat to the stem rust fungus *Puccinia*.

Reduced light intensity generally increases the susceptibility of plants to virus infections. Plants kept in the dark for 1 or 2 days before sap inoculation with a virus produce more local lesions (i.e., infections) than plants kept in the normal light–dark regime. This has become a routine procedure in many laboratories. Generally, keeping plants in the dark affects the sensitivity of plants to virus infection if it precedes inoculation with the virus, but it seems to have little or no effect on symptom development if it occurs after inoculation. However, low light intensities following inoculation tend to mask the symptoms of some diseases. In these diseases, symptoms are much more severe when the plants are grown in normal light than when they are shaded.

EFFECT OF SOIL PH AND SOIL STRUCTURE

The pH of the soil is important in the occurrence and severity of plant diseases caused by certain soilborne pathogens. For example, the clubroot of crucifers caused by *Plasmodiophora brassicae* is most prevalent and severe at about pH 5.7, whereas its development drops sharply between pH 5.7 and 6.2 and is completely checked at pH 7.8. On the contrary, the common scab of potato caused by *S. scabies* can be severe from pH 5.2 to 8.0 or above, but its development drops sharply below pH 5.2. It is obvious that such diseases are most serious in areas in which soil pH favors the particular pathogen. In these and many other diseases, the effect of soil acidity (pH) seems to be principally on the pathogen. In some diseases, however, a weakening of the host through altered nutrition that is induced by the soil acidity may affect the incidence and severity of the disease.

Soil factors other than pH may also influence the development of plant diseases. For example, the cotton root rot fungus (*Phymatotrichopsis omnivora*) affects many hosts, e.g., peach trees (Fig. 7-11A), and grows best at high pH (pH 7.2–8.0). The fungus, however, exists only in the southwestern United States and northern Mexico (Fig. 7-11B), where the soils contain relatively high concentrations of calcium carbonate.

EFFECT OF HOST–PLANT NUTRITION

Nutrition affects the rate of growth and the state of readiness of plants to defend themselves against pathogenic attack.

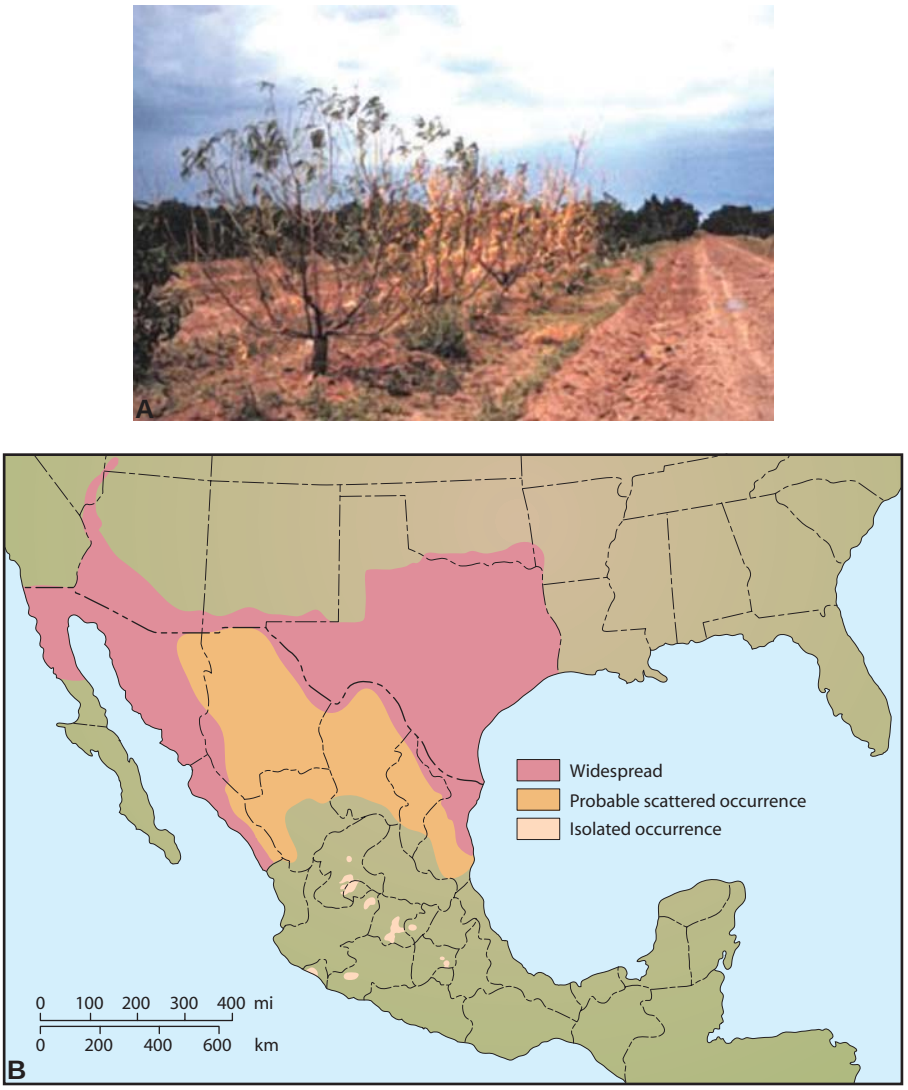

FIGURE 7-11 (A) Root rot and death of peach trees caused by the cotton root rot fungus *Phymatotrichopsis omnivora*. (B) Distribution of the cotton root rot fungus in North America is limited to areas with soils high in calcium carbonate (and high pH) and high temperatures. [Photographs courtesy of (A) R. B. Hines, New Mexico State University and (B) R. G. Percy (1983). *Plant Dis.* **67**, 981–983.]

Nitrogen abundance results in the production of young, succulent growth, a prolonged vegetative period, and delayed maturity of the plant. These effects make the plant more susceptible to pathogens that normally attack such tissues and for longer periods. Conversely, plants suffering from a lack of nitrogen are weaker, slower growing, and faster aging. Such plants, therefore, are susceptible to pathogens that are best able to attack weak, slow-growing plants. It is known, for example, that fertilization with large amounts of nitrogen increases the susceptibility of pear to fire blight (*Erwinia amylovora*) and of wheat to rust (*Puccinia*) and powdery mildew (*Erysiphe*). It has also been shown that *Cercospora* diseases of cereals, such as corn gray leaf spot, rice brown leaf spot, and the Sigatoka disease of banana, increase in severity with increasing nitrogen fertilization. The reduced availability of nitrogen may increase the susceptibility of tomato to *Fusarium* wilt, of many solanaceous plants to *Alternaria solani* early blight and *Ralstonia solanacearum* wilt, of sugar beets to *Sclerotium rolfsii*, and of most seedlings to *Pythium* damping off.

It is possible, however, that it is not the amount of nitrogen but the form of nitrogen (ammonium or nitrate) that is available to the host or pathogen that affects disease severity or resistance. Of numerous root rots, wilts, foliar diseases, and so on treated with either form of nitrogen, almost as many decreased or increased

in severity when treated with a source of ammonium nitrogen as did when treated with a source of nitrate nitrogen. Each form of nitrogen, however, had exactly the opposite effect on a disease (i.e., decrease or increase in severity) than the other form of nitrogen. For example, *Fusarium* spp., *P. brassicae, S. rolfsii*, *Pyrenochaeta lycopersici*, and the diseases they cause (root rots and wilts, clubroot of crucifers, damping off and stem rots, and corky root rot, respectively) increase in severity when an ammonium fertilizer is applied (Fig. 7-12). Alternatively, *P. omnivora, Gaeumannomyces graminis*, and *S. scabies*, and the diseases they cause (cotton root rot, take-all of wheat, and scab of potato, respectively) are favored by nitrate nitrogen. The effect of each nitrogen form appears to be associated with soil pH influences. Diseases increased by ammonium nitrogen are generally more severe at acid pH, whereas those increased by nitrate nitrogen are generally more severe at neutral to alkaline pH. Ammonium ions (NH_4^+) are absorbed by the roots through exchange with H^+ released by the roots to the surrounding medium, thus reducing soil pH.

Because of the profound effects of nitrogen on growth, nitrogen nutrition has been studied the most extensively in relation to disease development. Studies with other elements, however, such as phosphorus, potassium, and calcium, and also with micronutrients have indicated similar relationships between levels of the particular nutrients and susceptibility or resistance to certain diseases.

Phosphorus has been shown to reduce the severity of take-all disease of barley (caused by *G. graminis*) and potato scab (caused by *S. scabies*) but to increase the severity of cucumber mosaic virus on spinach and of leaf and glume blotch of wheat caused by *Septoria*. Phosphorus seems to increase resistance either by improving the balance of nutrients in the plant or by accelerating the maturity of the crop and allowing it to escape infection by pathogens that prefer younger tissues.

Potassium has also been shown to reduce the severity of numerous diseases, including stem rust of wheat, early blight of tomato, and gray leaf spot and stalk rot of corn, although high amounts of potassium seem to increase the severity of rice blast (caused by *Magnaporthe grisea*), corn gray leaf spot (caused by *Cercospora zeae-maydis*), and root knot (caused by the nematode *Meloidogyne incognita*). Potassium seems to have a direct effect on the various stages of pathogen establishment and development in the host and an indirect effect on infection by promoting wound healing. Potassium also increases resistance to frost injury and thereby reduces infection that commonly begins in frost-killed tissues. In addition, potassium delays maturity and senescence in some crops and during these periods

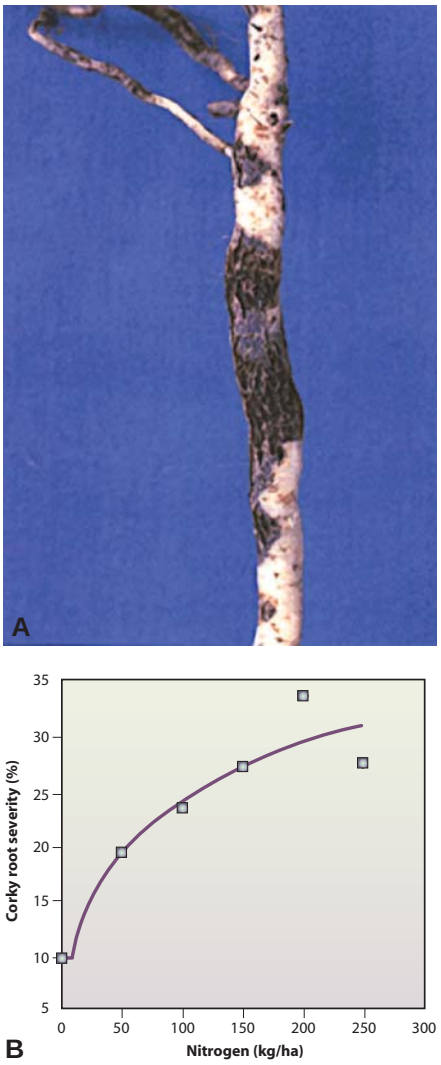

FIGURE 7-12 (A) Corky root of tomato caused by *Pyrenochaeta lycopersici*. (B) Effect of amount of nitrogen (ammonium nitrate) applied to the soil on the severity (percentage of root length infected) of corky root of tomato. [Photographs courtesy of (A) R. J. McGovern, University of Florida and (B) Worneh and van Bruggen (1994). *Phytopathology* **84**, 688–694.]

infection by certain facultative parasites can be severely damaging.

Calcium reduces the severity of several diseases caused by root and stem pathogens, such as the fungi *Rhizoctonia*, *Sclerotium*, and *Botrytis*, the wilt fungus *Fusarium oxysporum*, and the nematode *Ditylenclus dipsaci*, but it increases the black shank disease of tobacco (caused by *Phytophthora parasitica* var. *nicotianae*) and the common scab of potato (caused by *S. scabies*). The effect of calcium on disease resistance seems to result from its effect on the composition of cell walls and their resistance to penetration by pathogens.

A reduction in disease levels was also observed when levels of certain micronutrients were increased. For example, application of **iron** to the soil reduced Verticillium wilts of mango and of peanuts. Foliar applications of iron compounds reduced the severity of silver leaf of deciduous fruit trees (caused by *Chondrostereum purpureum*). **Copper** applications to the soil significantly reduced take-all and ergot diseases (caused by the fungi *G. graminis* and *Claviceps purpurea*, respectively), as well as stem melanosis (caused by the bacterium *Pseudomonas chicorii*) in wheat and barley. Similarly, applications of **manganese** reduced potato scab and late blight of potato and stem rot (caused by *Sclerotinia sclerotiorum*) of pumpkin seedlings, but the addition of **magnesium** increased the severity of corn leaf blight caused by *Cochliobolus heterostrofus*, whereas applications of molybdenum reduced late blight of potato and *Ascochyta* blight of beans and peas. The severity of other diseases, however, was raised by the presence of higher levels of these micronutrients, e.g., Fusarium wilt of tomato by increased iron or manganese and tobacco mosaic of tomatoes by increased manganese.

In recent years, the addition of **silicon** to the soil or to the nutrient solution supplied to greenhouse plants has been shown to reduce diseases. Field applications of various grades of silicon increased the amount of silicon taken up by the plants (Fig. 7-13A) and reduced the amount of disease in rice such as brown spot of rice

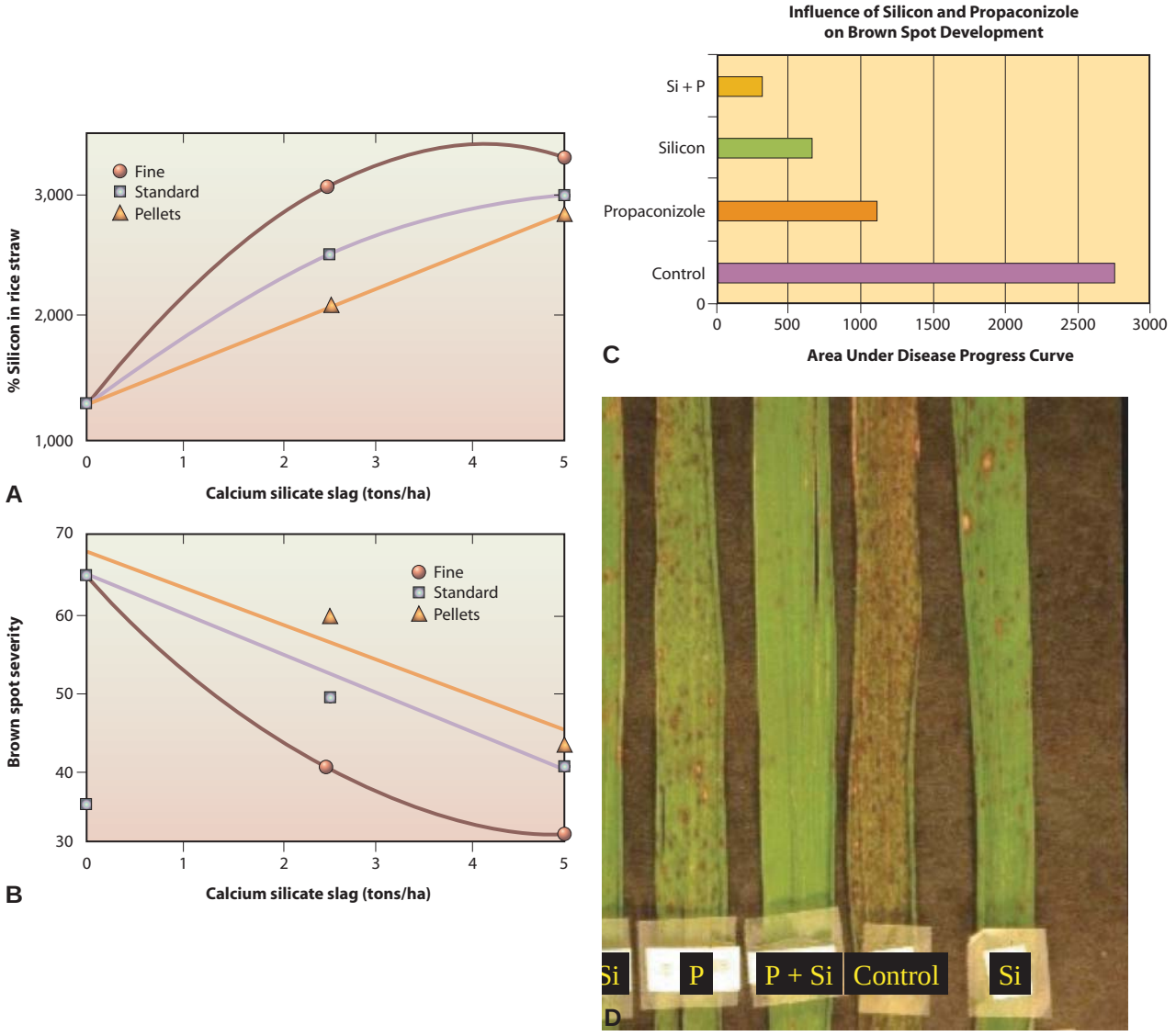

FIGURE 7-13 (A) Relationship between calcium silicate slag grades and quantity to the concentration of silicon in rice straw. (B) Reduction of severity of the brown spot disease of rice caused by the fungus *Cochliobolus miyabeanus*. (C and D) Comparison of brown spot reduction by silicon and fungicide application. [Courtesy of (A and B) Datnoff (1992). *Plant Dis.* **76**, 1011–1013 and (C and D) L. E. Datnoff, University of Florida.]

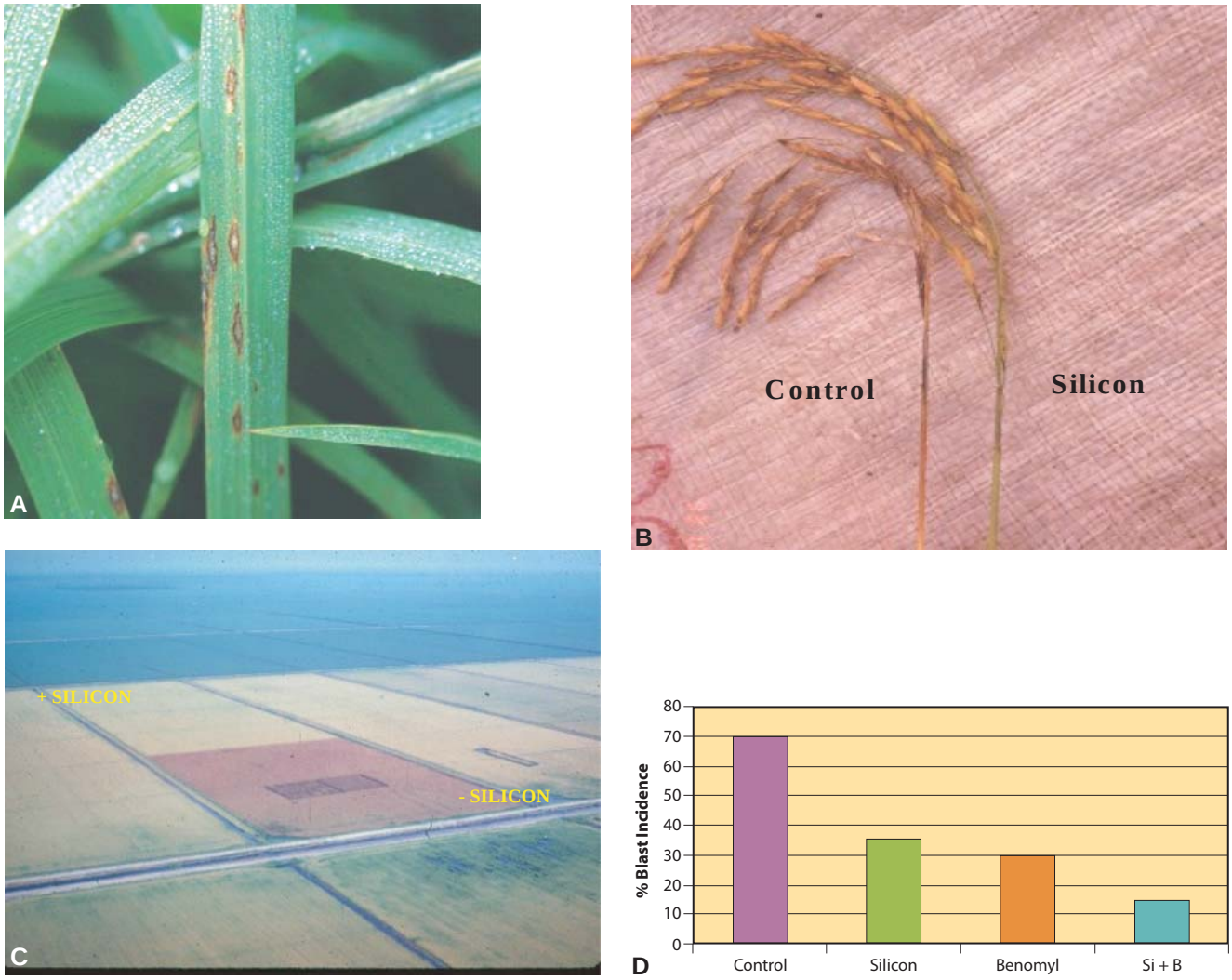

FIGURE 7-14 (A) Characteristic lesions on rice leaf (A) and panicle neck rot and blast (B) caused by the rice blast fungus *Magnaporthe grisea*. (C) Aerial photograph of a rice field, the distal half of which (+silicon) received 3 tons/acre calcium silicate slag, while the proximal half (–silicon) did not. Note the brown discoloration of the proximal half due to infection by the rice blast disease and some rice brown spot, while the distal half receiving silicon shows little or no browning of plants. (D) Graph showing the effect of silicon alone or in combination with the fungicide benomyl on the incidence of rice blast in comparison with an untreated rice field (control). (Courtesy of L. E. Datnoff, University of Florida.)

(Figs. 7-13B and 7-13C) caused by *Cochliobolus miyabeanus,* of rice blast (Fig. 7-14) caused by the fungus *M. grisea*, and of rice sheath blight caused by *Rhizoctonia solani*. The addition of silicon to the soil reduced brown spot more than application of a fungicide (Figs. 7-13C and 7-13D), reduced rice blast comparable to that of a fungicide application (Figs. 7-14C and 7-14D), and reduced rice sheath blight by at least 50% not only in susceptible but also in the resistant varieties (Figs. 7-15A and 7-15B). In greenhouse applications, silicon reduced disease levels, for example, of cucumber powdery mildew and cucumber root rot caused by the fungus *Sphaerotheca fuligena* and the oomycete *Pythium ultimum*, respectively, and of wheat powdery mildew caused by *Blumeria graminis* f. sp. *tritici*. In the latter, epidermal cells of silicon-treated plants produced specific defense reactions upon inoculation with the powdery mildew fungus, including the formation of papilla, production of callose, and release of phenolic compounds that accumulated along the cell wall and affected the integrity of the pathogen.

In diseases caused by phytoplasmas and spiroplasmas, such as maize bushy stunt and corn stunt, respectively, diseased plants took up less nutrients than healthy plants regardless of the level of availability of soil water, and spiroplasma-infected plants suppressed particularly the uptake of Mg from the soil.

In general, plants receiving a balanced nutrition, in which all required elements are supplied in appropriate amounts, are more capable of protecting themselves

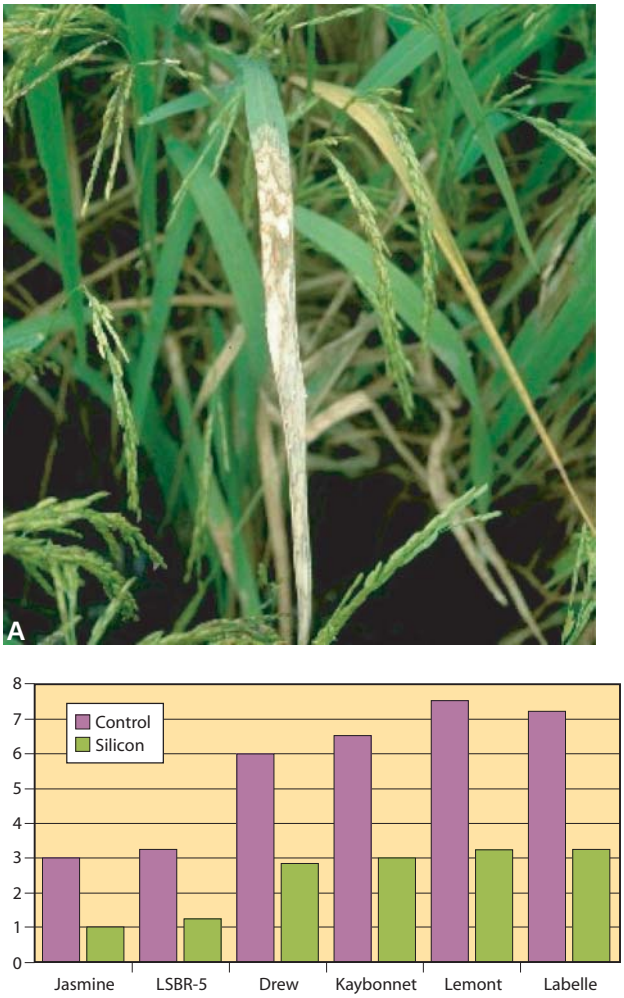

FIGURE 7-15 (A) Symptoms of rice sheath blight on rice leaves and stems caused by *Rhizoctonia solani*. (B) Graph showing the reduction of sheath blight severity by silicon added to fields planted to rice varieties of various resistances to sheath blight. Even the most resistant varieties benefited from the addition of silicon to the soil. (Courtesy of L. E. Datnoff, University of Florida.)

from new infections and of limiting existing infections than plants to which one or more nutrients are supplied in excessive or deficient amounts. However, even balanced nutrition may affect the development of a disease when the concentration of all the nutrients is increased or decreased beyond a certain range.

EFFECT OF HERBICIDES

Herbicide use is common and widespread in agriculture. In many cases, herbicides have been shown to increase the severity of certain diseases on crop plants, for example, of *R. solani* on sugar beets and cotton, *Fusarium* wilt of tomatoes and cotton, and *Sclerotium* stem rots of various crops. In other plant–pathogen combinations, herbicides appear to decrease disease, for example, *Aphanomyces euteiches* root rot of peas, *Pseudocercosporella herpotrichoides* foot rot of wheat, and *Phytophthora* collar rot of various crops. Herbicides apparently act on plant diseases either directly or indirectly. The direct effects may include stimulation or retardation of the growth of the pathogen or an increase or decrease in the susceptibility of the host. Indirect effects include an increase or decrease in the activity of soil microflora, elimination or selection of the pathogen by certain additional or alternate hosts, or alteration of the microclimate of the crop plant canopy (e.g., change in humidity).

EFFECT OF AIR POLLUTANTS

Air pollutants cause various types of direct symptoms on plants exposed to high levels of pollutants. In infectious plant diseases, both the plant and the pathogen are exposed to the same levels of pollutants, but it is not yet clear whether the presence of a particular pollutant causes an increase or a decrease in the severity of the disease caused by the pathogen alone. It appears, however, that some air pollutants, such as ozone, may affect a pathogen and sometimes the disease it causes. For example, with the rusts on oats and wheat, ozone reduces the growth of uredia and of hyphal growth and also the number of uredospores produced on ozone-injured leaves, whereas with the powdery mildew of barley, the rate of infection is reduced if the exposure to ozone is early but is increased if exposure occurs late. With nonobligate parasites, ozone may increase the percentage of diseased leaf area of wheat by *Drecslera* fungus, infection of potato leaves by *Botrytis* occurs only on ozone-injured leaves, and in the *Lophodermium* needle blight of pine, ozone exposure increases the severity of the needle blight. Similarly, the bacteria *Pseudomonas glycinea,* infecting soybean, and *Xanthomonas alfalfae,* infecting alfalfa, caused a smaller number of lesions on plants exposed to ozone than on unexposed ones.

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chapter eight

PLANT DISEASE EPIDEMIOLOGY

When a pathogen spreads to and affects many individuals within a population over a relatively large area and within a relatively short time, the phenomenon is called an epidemic. An epidemic has been defined as any increase of disease in a population. A similar definition of an epidemic is the dynamics of change in plant disease in time and space. The study of epidemics and of the factors that influence them is called epidemiology. Epidemiology is concerned simultaneously with populations of pathogens and host plants as they occur in an evolving environment, i.e., the classic disease triangle. As a result, epidemiology is also concerned with population genetics of host resistance and with the evolutionary potential of pathogen populations to produce pathogen races that may be more virulent to host varieties or more resistant to pesticides. Epidemiology, however, must also take into account other biotic and abiotic factors, such as an environment strongly influenced by human activity, particularly as it relates to disease management.

Plant disease epidemics, sometimes called epiphytotics, occur annually on most crops in many parts of the world. Most epidemics are more or less localized and cause minor to moderate losses. Some epidemics are kept in check naturally, e.g., by changes in weather conditions. Others are kept in check by chemical sprays and other control measures. Occasionally, however, some epidemics appear suddenly, go out of control, and become extremely widespread or severe on a particular plant species. Some plant disease epidemics, e.g., wheat rusts, southern corn leaf blight (Fig. 8-1), and grape downy mildew, have caused tremendous losses of produce over rather large areas. Others, e.g., chestnut blight (Fig. 1-8), Dutch elm disease, and coffee rust, have threatened to eliminate certain plant species from entire continents. Still others have caused untold suffering to humans. The Irish potato famine of 1845–1846 was caused by the Phytophthora late blight epidemic of potato, and the Bengal famine of 1943 was caused by the *Cochliobolus* (*Helminthosporium*) brown spot epidemic of rice.

THE ELEMENTS OF AN EPIDEMIC

Plant disease epidemics develop as a result of the timely combination of the same elements that result in plant disease: susceptible host plants, a virulent pathogen, and favorable environmental conditions over a relatively long period of time. Humans may unwittingly help initiate and develop epidemics through some of their activities, e.g., by topping or pruning plants in wet weather. More frequently, humans may stop the initiation and development of epidemics by using appropriate control measures under situations in which epidemics would almost certainly occur without human intervention. Thus, the chance of an epidemic increases when the susceptibility of the host and virulence of the pathogen are greater, as the environmental conditions approach the optimum level for pathogen growth, reproduction, and spread, and as the duration of all favorable combinations is prolonged or repeated.

To describe the interaction of the components of plant disease epidemics, the disease triangle, which is

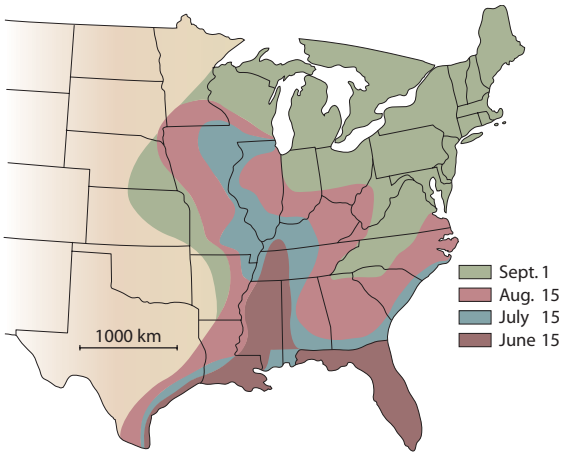

FIGURE 8-1 Development and northward spread of the southern corn leaf blight epidemic, caused by *Cochliobolus heterostrophus* (*Bipolaris maydis*), in the United States from June 15 to September 1, 1970. [From Zadoks and Schein (1979).]

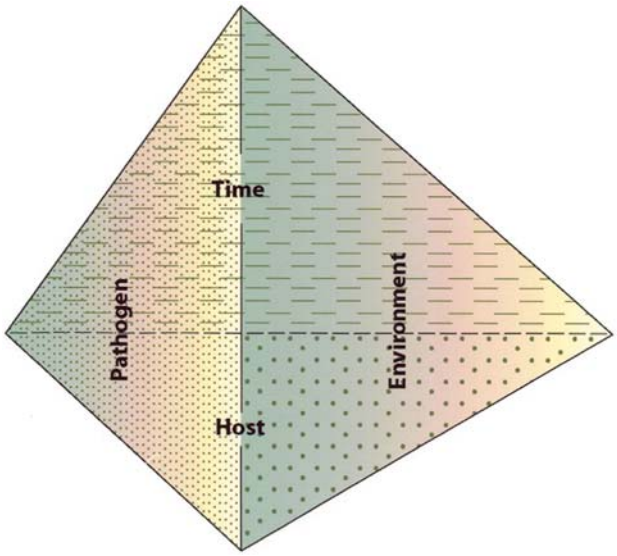

FIGURE 8-2 The disease tetrahedron.

discussed in Chapter 2 and describes the interaction of the components of plant disease, can be expanded to include time and humans. Indeed, the amount of each of the three components of plant disease and their interactions in the development of disease are affected by a fourth component: time. Both the specific point in time at which a particular event in disease development occurs and the length of time during which the event takes place affect the amount of disease. The interaction of the four components can be visualized as a tetrahedron, or pyramid, in which each plane represents one of the components. This figure is referred to as the disease tetrahedron or disease pyramid (Fig. 8-2). The effect of time on disease development becomes apparent when one considers the importance of the time of year (i.e., the climatic conditions and stage of growth when host and pathogen may coexist), the duration and frequency of favorable temperature and rains, the time of appearance of the vector, the duration of the infection cycle of a particular disease, and so on. If the four components of the disease tetrahedron could be quantified, the volume of the tetrahedron would be proportional to the amount of disease on a plant or in a plant population.

Disease development in cultivated plants is also influenced greatly by a fifth component: humans. Humans affect the kind of plants grown in a given area, the degree of plant resistance, the numbers planted, time of planting, and density of the plants. By the resistance of the particular plants they cultivate, humans also determine which pathogens and pathogen races will predominate. By their cultural practices, and by the chemical and biological controls they may use, humans affect the amount of primary and secondary inoculum available to attack plants. They also modify the effect of environment on disease development by delaying or speeding up planting or harvesting, by planting in raised beds or in more widely spaced beds, by protecting plant surfaces with chemicals before rains, by regulating the humidity in produce storage areas, and so on. The timing of human activities in growing and protecting plants may affect various combinations of these components to a considerable degree, thereby affecting the amount of disease in individual plants and in plant populations greatly. The human component has sometimes been used in place of the component "time" in the disease tetrahedron, but it should be considered a distinct fifth component that influences the development of plant disease directly and indirectly.

In Fig. 8-3, host, pathogen, and environment are each represented by one of the sides of the triangle, time is represented as the perpendicular line arising from the center of the triangle and humans as the peak of the tetrahedron whose base is the triangle and height is the length of time. In this way, humans interact with and influence each of the other four components of an epidemic, thereby increasing or decreasing the magnitude of the epidemic. Sometimes, of course, humans themselves can be affected to a greater or lesser extent by plant disease epidemics.

HOST FACTORS THAT AFFECT THE DEVELOPMENT OF EPIDEMICS

Several internal and external factors of particular host plants play an important role in the development of epidemics involving those hosts.

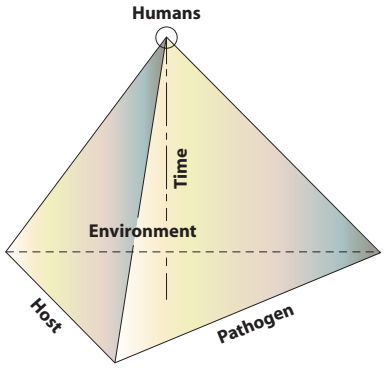

FIGURE 8-3 Schematic diagram of the interrelationships of the factors involved in plant disease epidemics.

Levels of Genetic Resistance or Susceptibility of the Host

Obviously, host plants carrying race-specific (vertical) resistance do not allow a pathogen to become established in them, and thus no epidemic can develop (Fig. 8-4). Host plants carrying partial (horizontal) resistance will probably become infected, but the rate at which the disease and the epidemic will develop depends on the level of resistance and the environmental conditions. Susceptible host plants lacking genes for resistance against the pathogen provide the ideal substrate for establishment and development of new infections. Therefore, in the presence of a virulent pathogen and a favorable environment, susceptible hosts favor the development of disease epidemics.

Degree of Genetic Uniformity of Host Plants

When genetically uniform host plants, particularly with regard to the genes associated with disease resistance, are grown over large areas, a greater likelihood exists that a new pathogen race will appear that can attack their genome and result in an epidemic. This phenomenon has been observed repeatedly, for example, in the *Cochliobolus* (*Helminthosporium*) blight on Victoria oats and in southern corn leaf blight (Fig. 8-1) on corn carrying Texas male-sterile cytoplasm. For similar reasons of genetic uniformity, the highest rates of epidemic development generally occur in vegetatively propagated crops, intermediate rates in self-pollinated crops, and the lowest rates in cross-pollinated crops. This explains why most epidemics develop rather slowly in natural populations, where plants of varying genetic makeup are intermingled.

Type of Crop

In diseases of annual crops, such as corn, vegetables, rice, and cotton, and in foliar, blossom, or fruit diseases of trees and vines, epidemics generally develop much more rapidly (usually in a few weeks) than they do in diseases of branches and stems of perennial woody crops such as fruit and forest trees. Some epidemics of fruit and forest trees, e.g., tristeza in citrus, pear decline, Dutch elm disease, and chestnut blight, take years to develop.

Age of Host Plants

Plants change in their reaction (susceptibility or resistance) to disease with age. The change of resistance with age is known as ontogenic resistance. In some plant–pathogen combinations, e.g., *Pythium* damping off and root rots, downy mildews, peach leaf curl, systemic smuts, rusts, bacterial blights, and viral infections, the hosts (or their parts) are susceptible only during the

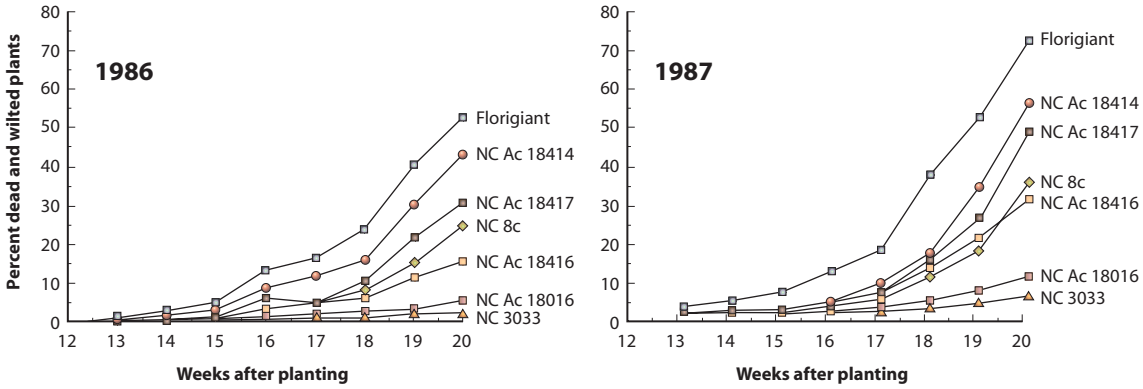

FIGURE 8-4 Development of *Cylindrocladium* black rot, caused by the fungus *C. crotalariae*, on susceptible (Florigiant), resistant (NC3033), and intermediate peanut varieties. The various genotypes maintain their resistance rankings in both years (1986, 1987) and at all inoculum density levels tested. [From Culbreath *et al.* (1991).]

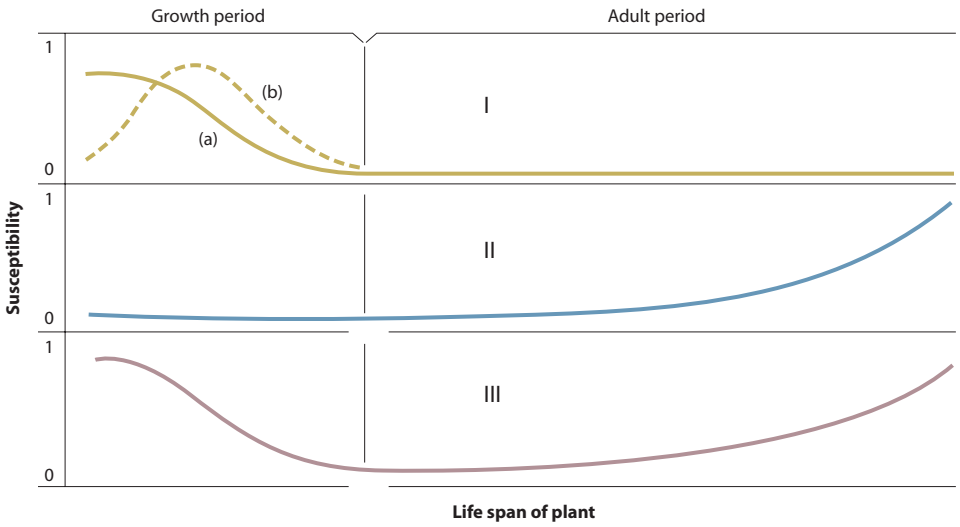

FIGURE 8-5 Change of susceptibility of plant parts with age. In pattern I, plants are susceptible only in the stages of maximum growth (Ia) or in the earliest stages of growth (Ib). In pattern II, plants are susceptible only after they reach maturity, and susceptibility increases with senescence. In pattern III, plants are susceptible while very young and again after they reach maturity. [After Populer (1978).]

growth period and become resistant during the adult period (adult resistance) (Figs. 8-5Ia and 8-5Ib). With several diseases, such as rusts and viral infections, plant parts are actually quite resistant to infection while still very young, become more susceptible later in their growth, and then become resistant again before they are fully expanded (Figs. 8-5, pattern Ib, and 8-6). In other diseases, such as infections of blossoms or fruit by *Botrytis*, *Penicillium*, *Monilinia*, and *Glomerella*, and in all postharvest infections, plant parts are resistant during growth and the early adult period but become susceptible near ripening (Fig. 8-5II). In still other diseases, such as potato late blight (caused by *Phytopthora infestans*) and tomato early blight (caused by *Alternaria solani*), a stage of juvenile susceptibility during the growth period of the plant is followed by a period of relative resistance in the early adult stage and then susceptibility after maturity (Fig. 8-5III).

Apparently then, depending on the particular plant–pathogen combination, the age of the host plant at the time of arrival of the pathogen may affect considerably the development of infection and of an epidemic.

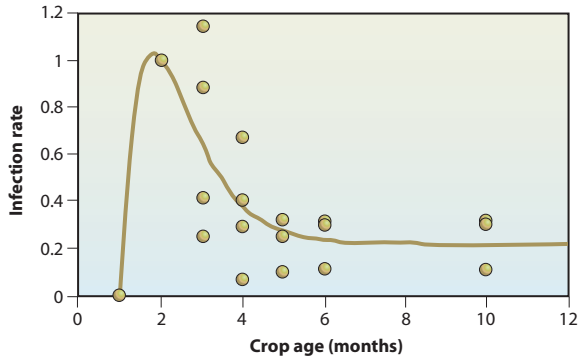

FIGURE 8-6 Effect of crop age on rate of infection. Cassava plantings of different ages exposed to the whitefly-transmitted African cassava mosaic geminivirus show increased resistance to infection as they age. [From Fargette and Vie (1994). *Phytopathology* **84**, 378–382.]

PATHOGEN FACTORS THAT AFFECT DEVELOPMENT OF EPIDEMICS

Levels of Virulence

Virulent pathogens capable of infecting the host rapidly ensure a faster production of larger amounts of inoculum, and, thereby, disease, than pathogens of lesser virulence.

Quantity of Inoculum near Hosts

The greater the number of pathogen propagules (bacteria, fungal spores and sclerotia, nematode eggs, virus-infected plants, etc.) within or near fields of host plants, the more inoculum reaches the hosts and at an earlier time, thereby increasing the chances of an epidemic greatly (Fig. 8-7).

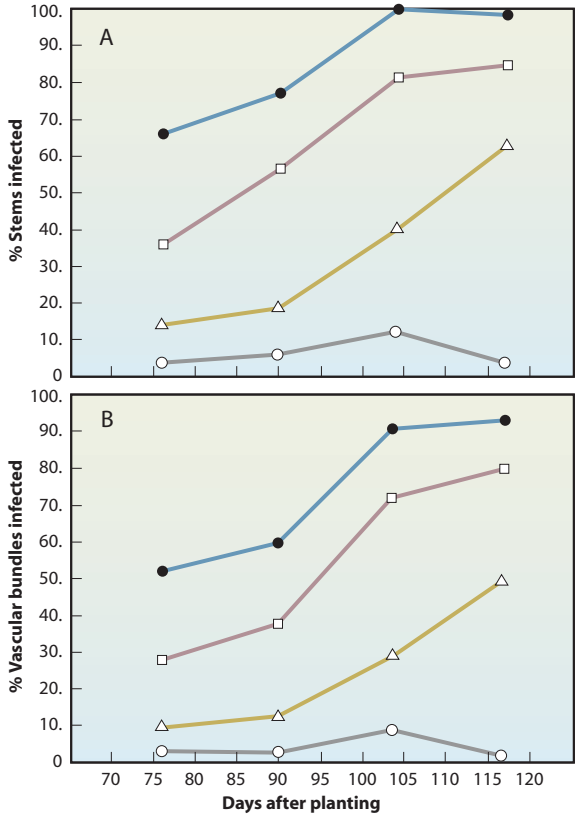

FIGURE 8-7 Effect of amount of soil inoculum of *Verticillium dahliae* on the amount of vascular wilt on potato plants at various dates after planting. Disease is expressed as a percentage of stems (A) and of main vascular bundles (B) infected at the base of the plants. ○, no pathogen detected; △, 1–5 propagules per gram (ppg); □, 6–10 ppg; and ●, more than 10 ppg. [From Nicot and Rouse (1987). *Phytopathology* 77, 1346–1355.]

Type of Reproduction of the Pathogen

All pathogens produce many offspring. Some of them, such as most fungi, bacteria, and viruses, produce a great many offspring, while a few fungi, all nematodes, and all parasitic plants produce relatively small numbers of offspring. Some plant pathogenic fungi, bacteria, and viruses have short reproduction cycles and therefore are polycyclic, i.e., they can produce many generations in a single growing season. Polycyclic pathogens include fungi that cause rusts, mildews, and leaf spots and are responsible for most of the sudden, catastrophic plant disease epidemics in the world. Some soil fungi, such as *Fusarium* and *Verticillium*, and most nematodes usually have one to a few (up to four) reproductive cycles per growing season. For these latter pathogens, the smaller number of offspring and, especially, the conditions of their dispersal limit their potential to cause sudden and widespread epidemics in a single season. Nevertheless, they often cause localized, slower developing epidemics

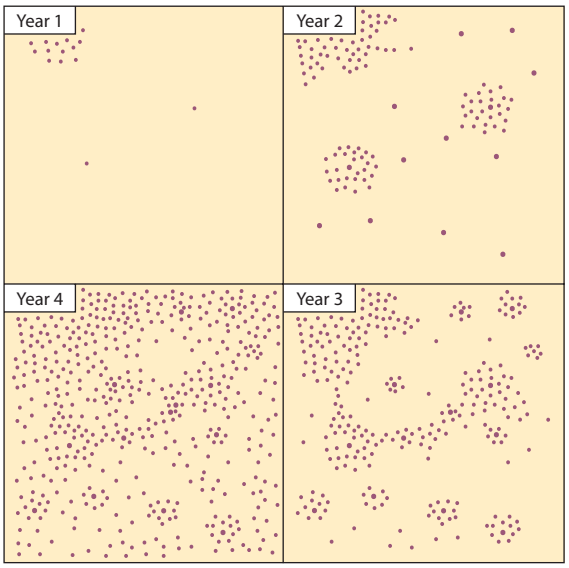

FIGURE 8-8 Schematic representation of a polyetic epidemic caused in a crop in a field by a soil pathogen over a 4-year period.

(Fig. 8-8). Several pathogens, such as the smuts and several short-cycle rusts, require an entire year to complete a life cycle (monocyclic pathogens) and can therefore cause only one series of infections per year. In such monocyclic diseases, the inoculum builds up from one year to the next, and the epidemic is usually polyetic, i.e., it develops over several years. Similarly, epidemics caused by pathogens that require more than a year to complete a reproductive cycle are slow to develop. Examples are cedar-apple rust (2 years), white pine blister rust (3–6 years), and dwarf mistletoe (5–6 years). As a result of overlapping of the polyetic generations, however, even such pathogens each year produce more inoculum and cause a series of infections that lead to long-term epidemics.

Ecology of the Pathogen

Some pathogens, such as most fungi and all parasitic higher plants, produce their inoculum (spores and seeds, respectively) on the surface of the aerial parts of the host. From there, spores and seeds can be dispersed with ease over a range of distances and can cause widespread epidemics. Other pathogens, such as vascular fungi and bacteria, mollicutes, viruses, and protozoa, reproduce inside the plant. In this case, spread of the pathogen is rare or impossible without the help of vectors. Therefore, such pathogens can cause epidemics only when vectors are plentiful and active. Still other pathogens, such as soilborne fungi, bacteria, and nematodes, produce their inoculum on infected plant parts in

the soil, within which the inoculum disperses slowly and presents little danger for sudden or widespread epidemics.

Mode of Spread of the Pathogen

The spores of many plant pathogenic fungi, such as those causing rusts, mildews, and leaf spots, are released into the air and can be dispersed by air breezes or strong winds over distances varying from a few centimeters up to several kilometers. These kinds of fungi are responsible for the most frequent and most widespread epidemics. In terms of their ability to cause sudden and widespread epidemics, the next most important group of pathogens includes those whose inoculum is carried by airborne vectors. Many of the viruses are transmitted by aphids, whiteflies, and some other insects. Mollicutes and fastidious bacteria are transmitted by leafhoppers, plant hoppers, or psyllids. Some fungi (such as the cause of Dutch elm disease), bacteria (such as the cause of bacterial wilt of cucurbits), and even nematodes (such as the cause of pine wilt disease) are disseminated primarily by beetles. Pathogens that are transmitted by wind-blown rain (primarily fungi causing diseases such as anthracnoses and apple scab, and most bacteria) are almost annually responsible for severe but somewhat localized epidemics within a field, a country, or a valley. Pathogens carried with the seed or other vegetative propagative organs (such as tubers or bulbs) are often placed in the midst of susceptible plants, but their ability to cause epidemics depends on the effectiveness of their subsequent transmission to new plants. Finally, pathogens present in and spreading through the soil, because of the physical restrictions imposed by the soil, are generally unable to cause sudden or widespread epidemics but often cause local, slow-spreading diseases of considerable severity (Fig. 8-9A). When such primarily soil fungi, however, also produce wind-disseminated spores, the latter can spread considerable distances and can cause epidemics destructive over considerable areas (Fig. 8-9B).

ENVIRONMENTAL FACTORS THAT AFFECT DEVELOPMENT OF EPIDEMICS

The majority of plant diseases occur wherever the host is grown but, usually, do not develop into severe and widespread epidemics. The concurrent presence in the same areas of susceptible plants and virulent pathogens does not always guarantee numerous infections, much less the development of an epidemic. This fact dramatizes the controlling influence of the environment on the development of epidemics. The environment may affect the availability, growth stage, succulence, and genetic susceptibility of the host plants. It may also affect the survival, vigor, rate of multiplication, sporulation, and ease, direction, and distance of dispersal of the pathogen, as well as the rate of spore germination and penetration. In addition, the environment may affect the number and activity of the vectors of the pathogen. The most important environmental factors that affect the development of plant disease epidemics are moisture, temperature, and the activities of humans in terms of cultural practices and control measures.

Moisture

As discussed in Chapter 7, abundant, prolonged, or repeated high moisture, whether in the form of rain, dew, or high humidity, is the dominant factor in the development of most epidemics of diseases caused by oomycetes

FIGURE 8-9 (A) Lettuce heads infected by soilborne sclerotia of *Sclerotinia sclerotiorum*. (B) Large field of lettuce heads killed by infections with airborne ascospores of the same fungus. [Photographs courtesy K. V. Subbarao, *Plant Dis*. 82: 1068–1078 (1998)].

and fungi (blights, downy mildews, leaf spots, rusts, and anthracnoses), bacteria (leaf spots, blights, soft rots), and nematodes. Moisture not only promotes new succulent and susceptible growth in the host, but, more importantly, it increases sporulation of fungi (Figs. 7-6A and 7-8) and multiplication of bacteria. Moisture facilitates spore release by many fungi (Figs. 7-7 and 7-9) and the oozing of bacteria to the host surface, and it enables spores to germinate and zoospores, bacteria, and nematodes to move. The presence of high levels of moisture allows all these events to take place constantly and repeatedly and leads to epidemics. In contrast, the absence of moisture for even a few days prevents all of these events from taking place so that epidemics are interrupted or stopped completely. Some diseases caused by soilborne pathogens, such as *Fusarium* and *Streptomyces*, are more severe in dry than in wet weather, but such diseases seldom develop into important epidemics. Epidemics caused by viruses and mollicutes are affected only indirectly by moisture, primarily by the effect that higher moisture has on the activity of the vector. Moisture may increase the activity of some vectors, as happens with the fungal and nematode vectors of some viruses, or it may reduce the activity of the vectors, as happens with the aphid, leafhopper, and other insect vectors of some viruses and mollicutes. The activity of these vectors is reduced drastically in rainy weather.

Temperature

Epidemics are sometimes favored by temperatures higher or lower than the optimum for the plant because they reduce the plant's level of partial resistance. At certain levels, temperatures may even reduce or eliminate the race-specific resistance of host plants. Plants growing at such temperatures become "stressed" and predisposed to disease, provided the pathogen remains vigorous.

Low temperature reduces the amount of inoculum of oomycete fungi, bacteria, and nematodes that survives cold winters. High temperature reduces the inoculum of viruses and mollicutes that survives hot summer temperatures. In addition, low temperatures reduce the number of vectors that survive the winter. Low temperatures occurring during the growing season can reduce the activity of vectors.

The most common effect of temperature on epidemics, however, is its effect on the pathogen during the different stages of pathogenesis, i.e., spore germination (Figs. 7-8 and 7-9) or egg hatching, host penetration, pathogen growth (Figs. 7-3 and 7-4) or reproduction, invasion of the host, and sporulation (Fig. 7-5). When temperature stays within a favorable range for each of these stages, a polycyclic pathogen can complete its infection cycle within a very short time (usually in a few days). As a result, polycyclic pathogens can produce many infection cycles within a growing season. Because the amount of inoculum is multiplied manyfold (perhaps 100 times or more) with each infection cycle and because some of the new inoculum is likely to spread to new plants, more infection cycles result in more plants becoming infected by more and more pathogens, thus leading to the development of a severe epidemic.

In reality, moisture and temperature must be favorable and act together in the initiation and development of the vast majority of plant diseases and plant disease epidemics.

EFFECT OF HUMAN CULTURAL PRACTICES AND CONTROL MEASURES

Many activities of humans have a direct or indirect effect on plant disease epidemics, some of them favoring and some reducing the frequency and the rate of epidemics.

Site Selection and Preparation

Low-lying and poorly drained and aerated fields, especially if near other infected fields, tend to favor the appearance and development of epidemics.

Selection of Propagative Material

The use of seed, nursery stock, and other propagative material that carries various pathogens increases the amount of initial inoculum within the crop and favors the development of epidemics greatly. The use of pathogen-free or treated propagative material can reduce the chance of epidemics greatly.

Cultural Practices

Continuous monoculture, large acreages planted to the same variety of crop, high levels of nitrogen fertilization, no-till culture, dense plantings (Fig. 8-10), overhead irrigation, injury by herbicide application, and poor sanitation all increase the possibility and severity of epidemics.

Disease Control Measures

Chemical sprays, cultural practices (such as sanitation and crop rotation), biological controls (such as using

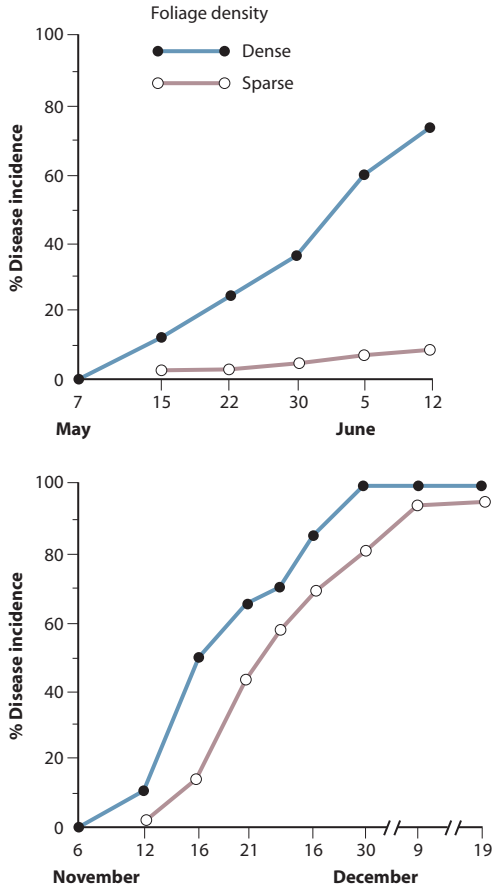

FIGURE 8-10 Effect of foliage density on development of *Phytophthora infestans* during a period of partly favorable weather (May–June) and of very favorable weather (November–December). [From Rotem and Ben-Joseph (1970). *Plant Dis. Rep.* **54**, 768–771.]

resistant varieties), and other control measures reduce or eliminate the possibility of an epidemic. Sometimes, however, certain controls, e.g., the use of a certain chemical or planting of a certain variety, may lead to selection of virulent strains of the pathogen that either are resistant to the chemical or can overcome the resistance of the variety and thus lead to epidemics.

Introduction of New Pathogens

The ease and frequency of worldwide travel have also increased the movement of seeds, tubers, nursery stock, and other agricultural goods. These events increase the possibility of introducing pathogens into areas where the hosts have not had a chance to evolve resistance to these pathogens. Such pathogens frequently lead to severe epidemics. Examples are chestnut blight, Dutch elm disease, and citrus canker caused by the bacterium *Xanthomonas campestris* pv. *citri*.

MEASUREMENT OF PLANT DISEASE AND OF YIELD LOSS

When measuring disease, one is interested in measuring (1) the **incidence** of the disease, i.e., the number or proportion of plant units that are diseased (i.e., the number or proportion of plants, leaves, stems, and fruit that show any symptoms) in relation to the total number of units examined; (2) the **severity** of the disease, i.e., the proportion of area or amount of plant tissue that is diseased; and (3) the **yield loss** caused by the disease, i.e., the proportion of the yield that the grower will not be able to harvest because the disease destroyed it directly or prevented the plants from producing it (the yield loss is the difference between **attainable yield** and **actual yield**).

Measuring disease incidence is relatively quick and easy, and this measurement is the one that is used commonly in epidemiological studies to measure the spread of a disease through a field, region, or country. In a few cases, such as cereal smuts, neck blast of rice, brown rot of stone fruits, and the vascular wilts of annuals, disease incidence has a direct relationship to the severity of the disease and yield loss because each diseased plant or fruit is a total loss. However, in many other diseases (such as most leaf spots, root lesions, and rusts) in which plants are counted as diseased whether they are exhibiting a single lesion or hundreds of lesions, disease incidence may have little relationship to the severity of the disease or to yield loss. Although severity and yield loss are of much greater importance to the grower than disease incidence, their measurement is more difficult and, in some cases, not possible until too late in the development of an epidemic.

Disease severity is usually expressed as the percentage or proportion of plant area or fruit volume destroyed by a pathogen (Figs. 8-11 and 8-12). More often, disease assessment scales from 0 to 10 or 1 to 4 are used to express the relative proportions of affected tissue at a particular point in time. Yield loss due to disease is measured at a specific growth stage, from sequential disease assessments at several stages of a crop's growth, or by determining the **area under a disease progress curve** (**AUDPC**), i.e., the area between the disease progress curve and the *X* axis of the graph. The area under the disease progress curve is used to summarize the progress of disease severity and is calculated by a formula that takes into account the number of times the disease severity was evaluated, the disease severity at each evaluation time, and the time duration of the epidemic.

Yield loss almost always results in **economic loss** from disease. Economic loss occurs whenever economic returns from the crop decrease because of reduced yields

(Fig. 8-13), because of the cost of agricultural activities undertaken to reduce damage to the crop, or both. In managing plant diseases, however, the grower can justify applying disease control measures only when the incremental costs of control are generally smaller than the increase in crop returns. The level of disease, i.e., the amount of plant damage, at which control costs just equal incremental crop returns is called the **economic threshold** of the disease. The economic threshold of a crop–pathogen system varies with the tolerance level (**damage threshold**) of the crop, which depends on the growth stage of the crop when attacked, crop management practices, environment, shifts in pathogen virulence, and new control practices. The economic threshold also varies with changing commodity prices and control costs.

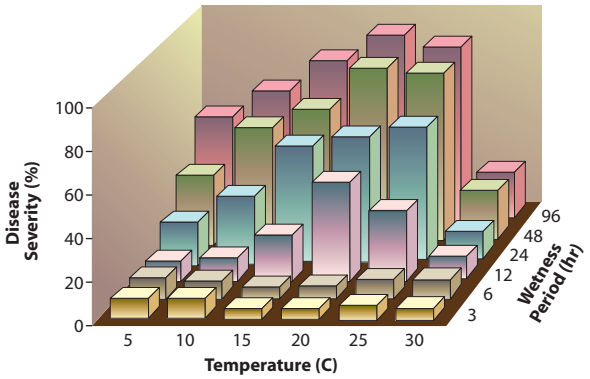

FIGURE 8-11 Development of *Ascochyta* blight of chickpea, caused by the fungus *Asochyta rabiei*, at different temperatures and leaf wetness durations. [From Trapero-Casas and Kaiser (1992). *Phytopathology* **82**, 589–596.]

PATTERNS OF EPIDEMICS

Interactions of the structural elements of epidemics, as influenced over time by factors of the environment and by human interference, are expressed in patterns and rates. The pattern of an epidemic in terms of the numbers of lesions, the amount of diseased tissue, or the numbers of diseased plants is given by a curve, called the **disease–progress curve**, that shows the progress of the epidemic over time. The point of origin and the shape of a disease–progress curve reveal information about the time of appearance and amount of inoculum, changes in host susceptibility during the growing period, recurrent weather events, and the effectiveness of cultural and control measures. Disease–progress curves, because they are affected by weather, variety, and so on, vary somewhat with location and time, but they are generally characteristic for some groups of diseases. For example, a saturation-type curve is characteristic for monocyclic diseases, a sigmoid curve is characteristic for polycyclic diseases, and a bimodal curve is characteristic for diseases affecting different organs (blossoms, fruit) of the plant (Fig. 8-14). Knowledge of disease–progress curves also allows disease forecasting and selection of the best control strategy for the particular disease and time.

The progress of an epidemic in space, in terms of changes in the number of lesions, the amount of diseased tissue, and the number of diseased plants as it spreads over distance, is called its spatial pattern, i.e., the arrangement of disease entities relative to each other and to the area of cultivation of the crop. Spatial patterns of epidemics are influenced by the dispersal of the pathogen, i.e., the process of movement of individuals of the pathogen in and out of the host population or

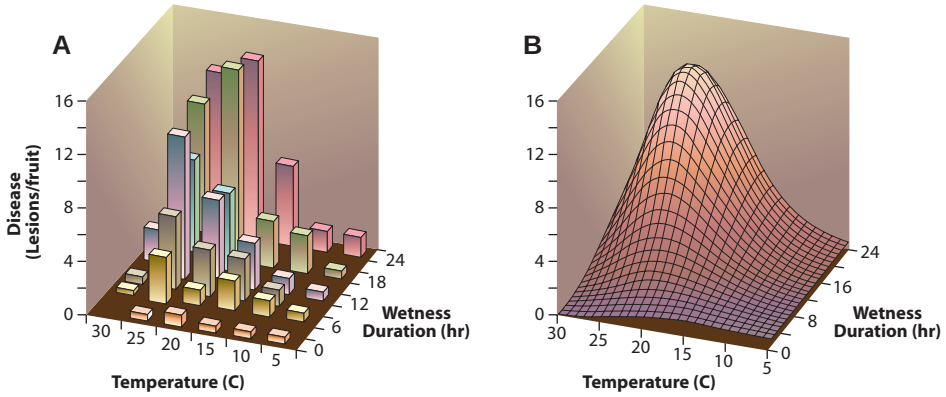

FIGURE 8-12 Severities of brown spot disease of pear, caused by the fungus *Stemphylium vesicarium*, at various combinations of temperature and wetness duration. (A) Experimental data. (B) Response surface diagram based on a model predicting the number of lesions per fruit at corresponding combinations. [From Montesinos *et al.* (1995). *Phytopathology* **85**, 586–592.]

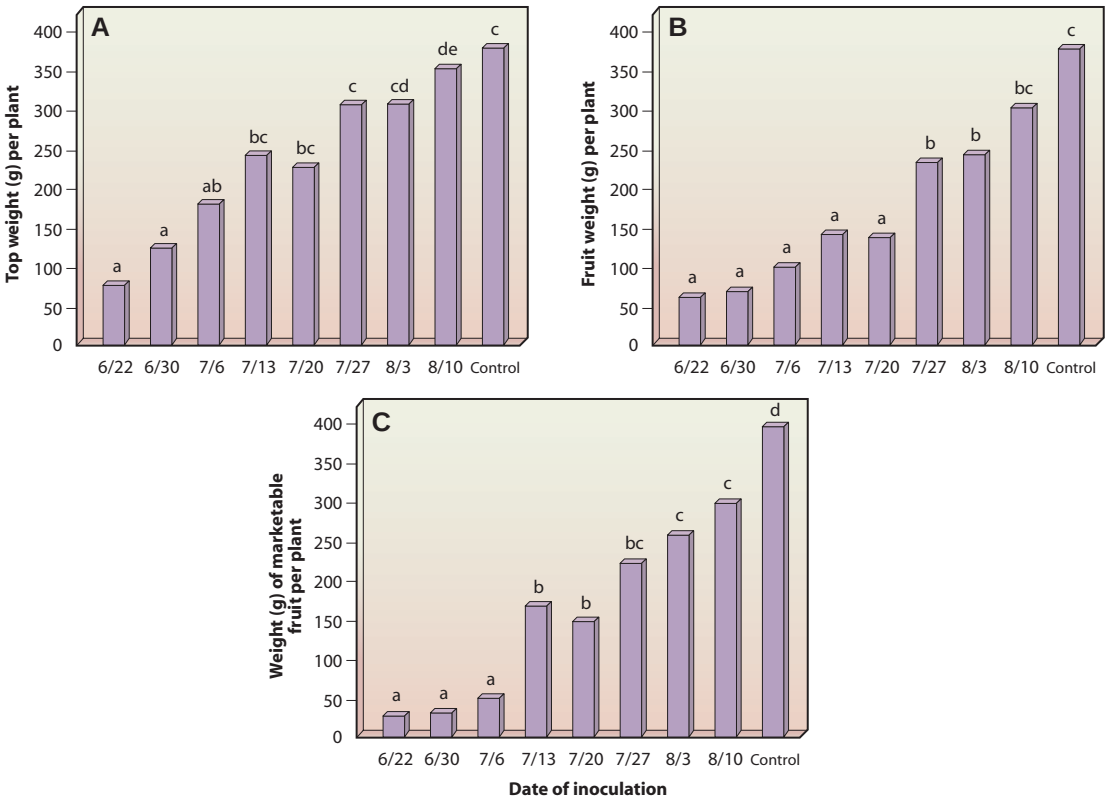

FIGURE 8-13 Average weight of tops minus fruit (A), of fruit (B), and of marketable fruit (C) of pepper plants inoculated with cucumber mosaic virus at different dates and of uninoculated control plants. [From Agrios *et al.* (1985). *Plant Dis.* **69**, 52–55.]

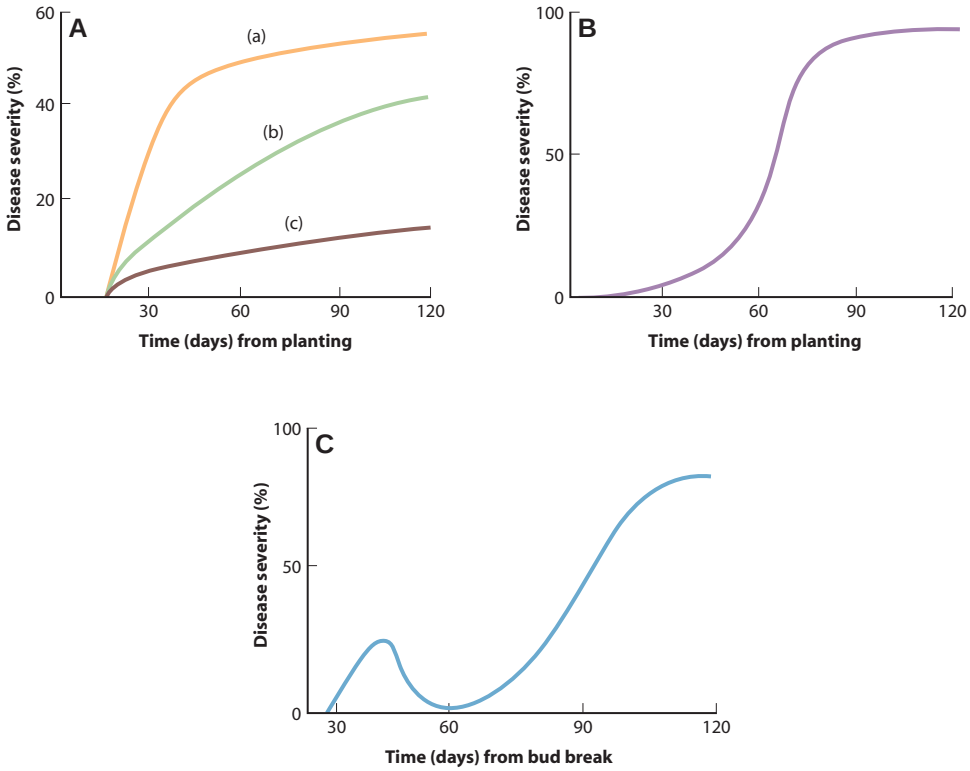

FIGURE 8-14 Schematic diagrams of disease–progress curves of some basic epidemic patterns. (A) Three monocyclic diseases of different epidemic rates. (B) Polycyclic disease, such as late blight of potato. (C) Bimodal polycyclic disease, such as brown rot of stone fruits, in which the blossoms and the fruit are infected at different, separate times.

population area, and is given by a curve that is called the **dispersal** or **disease–gradient curve**. Because the amount of disease is generally greater near the source of inoculum and decreases with increasing distance from the source, most disease–gradient curves are quite similar, at least in the early stages of the epidemic. The number of diseased plants and the severity of disease decrease steeply within short distances of the source and less steeply at greater distances until they reach zero or a low background level of occasional diseased plants (Fig. 8-15).

From data collected at various time intervals and used to plot the disease–progress curve of a disease (Fig. 8-16), one can obtain the epidemic rate of the disease, i.e., the rate of growth of the epidemic. The epidemic rate, generally designated *r*, is the amount of increase of disease per unit of time (per day, week, or year) in the plant population under consideration. The patterns of epidemic rates are given by curves called rate curves, and these curves are different for various groups of diseases (see Fig. 8-16). In some diseases, e.g., the late blight of potato, the rate curves are symmetrical (bell shaped) (Fig. 8-16A). In some diseases, e.g., in apple scab and most downy mildews and powdery mildews, the rate curves are asymmetrical, with the epidemic rate being greater early in the season (Fig. 8-16B) because of the greater susceptibility of young leaves. In still other diseases, the rate curves are asymmetrical, with the epidemic rate being greater late in the season (Fig. 8-16C). This is observed in the many diseases, e.g., *Alternaria* leaf blights and *Verticillium* wilts, that start slowly but accelerate markedly as host susceptibility increases late in the season.

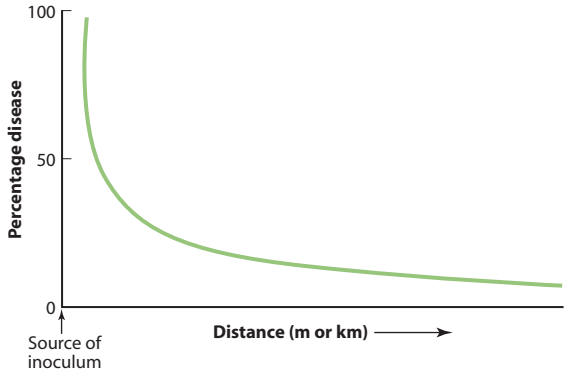

FIGURE 8-15 Schematic diagram of a disease–gradient curve. The percentage of disease and the scale for distance vary with the type of pathogen or its method of dispersal, being small for soilborne pathogens or vectors and larger for airborne pathogens.

COMPARISON OF EPIDEMICS

For better comparison of epidemics of the same disease at different times, different locations, or under different management practices or to compare different diseases, the patterns obtained for disease–progress curves and disease–gradient curves are frequently transformed mathematically into straight lines. The slopes of these lines can then be used to calculate epidemic rates.

In monocyclic diseases, the amount of inoculum does not increase significantly during the season. In such diseases, therefore, the rate of disease increase is affected only by the inherent ability of the pathogen to induce disease and by the ability of the environmental factors and cultural practices to influence host resistance and the virulence of the pathogen.

In contrast, the initial inoculum for diseases caused by polycyclic pathogens, although extremely important, has relatively less importance than the number of infection cycles in the final disease outcome (Fig. 8-17). Pathogens that have many infection cycles also have numerous opportunities to interact with the host. Therefore, the same factors mentioned earlier, namely the inherent ability of the pathogen to induce disease, environmental factors, host resistance, and cultural practices, have an opportunity to influence the dispersal, penetration, multiplication, size of lesion, rate of lesion formation, and rate and amount of sporulation, but they can do that not once but several times during the same growth season. The continuous or, sometimes, intermit-

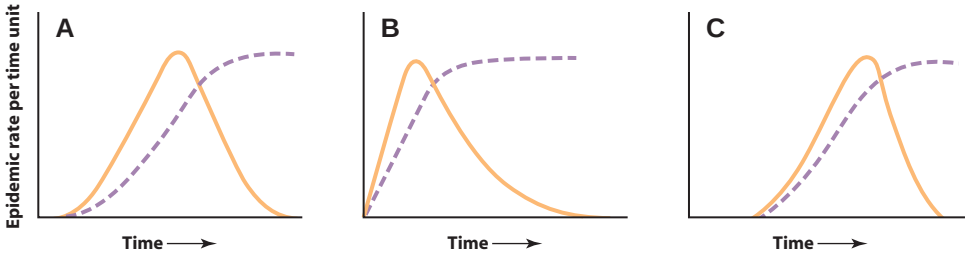

FIGURE 8-16 Schematic diagrams of epidemic rate curves of diseases with a symmetrical epidemic rate (A), with a high epidemic rate early in the season (B), and with a high epidemic rate late in the season (C). Dashed curves indicate possible disease–progress curves that may be produced in each case from the accumulated epidemic rate curves.

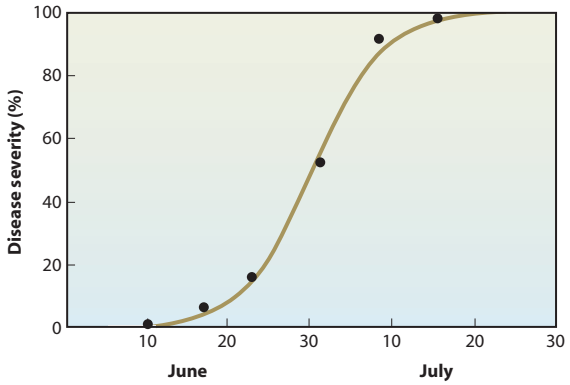

FIGURE 8-17 Predicted (—) and observed (●) disease progress curve of sunflower rust caused by the fungus *Puccinia helianthii*. [From Shtienberg and Vintal (1995). *Phytopathology* 85, 1388–1393.]

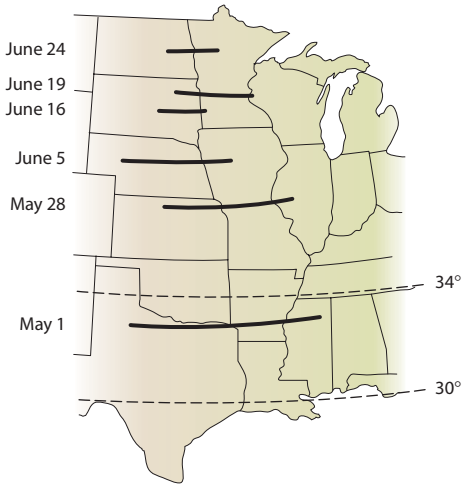

FIGURE 8-18 Normal annual advance of wheat stem rust across the United States. The fungus *Puccinia graminis tritici* generally overwinters south of the 30°N parallel and in trace amounts as far north as the 34°N parallel. In the spring it moves northward at the rates shown by the dates at left. [From Roelfs (1986).]

tent increase of the amount of inoculum and disease may result in highly variable infection rates for individual short-term intervals during the growth season, and quite variable epidemic rates for the entire season.

The epidemic rate for polycyclic diseases is usually calculated per day or per week rather than per year, which is the way it is calculated for monocyclic diseases. In general, the epidemic rate (r) for polycyclic diseases is much greater than the rate of epidemic increase (r_m) for monocyclic diseases. For example, the r_m for *Verticillium* wilt of cotton is 0.02 units per day and is 1.60 units per year for *Phymatotrichum* root rot of cotton. In contrast, the epidemic rate r for potato late blight is 0.3–0.5 units per day, is 0.3–0.6 units per day for wheat stem rust and 0.15 units per day for cucumber mosaic virus.

In addition to the epidemics caused by monocyclic and polycyclic pathogens, there are also polyetic epidemics. Pathogens causing polyetic epidemics are present for one year or more in the infected plant before they produce effective inoculum, e.g., some fungal wilts and viral and mollicute diseases of trees. Because of the perennial nature of their hosts, polyetic diseases behave basically as polycyclic diseases with a lower r. This happens because there are as many diseased trees and almost as much inoculum at the beginning of a year as at the end of the previous one, and both increase over the years, causing slower but just as severe epidemics. Some well-known polyetic epidemics are chestnut blight ($r = 0.3$–1.2 units per year) and elm yellows (phytoplasma) (0.6 units per year).

DEVELOPMENT OF EPIDEMICS

For a disease to become significant in a field, particularly if it is to spread over a large area and develop into a severe epidemic, specific combinations of environmental factors must occur either constantly or repeatedly, and at frequent intervals, over a large area. Even in a single, small field that contains the pathogen, plants almost never become severely diseased from just one set of favorable environmental conditions. It takes repeated infection cycles and considerable time before a pathogen produces enough individuals to cause an economically severe epidemic in the field (Fig. 8-1). Once large populations of the pathogen are available, however, they can attack, spread to nearby fields, and cause a severe epidemic in a very short time, often in just a few days.

A plant disease epidemic can occur in a garden, a greenhouse, or a small field, but "epidemic" generally implies the development and rapid spread of a pathogen on a particular kind of crop plant cultivated over a large area, such as a large field, a valley, a section of a country, the entire country, or even part of a continent (Figs. 8-18 and 8-19). Therefore, the first component of a plant disease epidemic is a large area planted to a genetically uniform crop plant, with the plants and the fields being close together. The second component of an epidemic is the presence or appearance of a virulent pathogen. Such cohabitations of host plants and pathogens occur, of course, daily in countless locations. Most of these, however, cause local diseases of varying severity, destroy crop plants to a limited extent, and do not develop into epidemics. Epidemics develop only when the combinations and progression of the right sets of conditions occur. These include appropriate temperature, moisture, and wind or insect vector coinciding with the susceptible stage or stages of the plant and with the production,

FIGURE 8-19 Annual occurrence of wheat stripe rust, caused by *Puccinia striiformis*, in northern China (shaded area). Gangu is the source region of the epidemic, and the other regions are in the dispersion area. The prevalent air currents are eastward in the fall and northward in the spring. [From Yang and Zeng (1992). *Phytopathology* **82**, 571–576.]

spread, inoculation, penetration, infection, and reproduction of the pathogen.

Thus, for an epidemic to develop, the small amount of original or primary inoculum of the pathogen must be carried by wind or vector to some of the crop plants as soon as they begin to become susceptible to that pathogen. The moisture and temperature must then be appropriate for germination or infection to take place. After infection, the temperature must be favorable for rapid growth and reproduction of the pathogen (short incubation period, short infection cycle) so that numerous new spores will appear as quickly as possible. The moisture (rain, fog, dew) then must be sufficient and should last long enough for the abundant release of spores. Winds of the proper humidity and velocity, blowing toward the susceptible crop plants, must then pick up the spores and carry them to the plants while the latter are still susceptible. Most plant disease epidemics spread from south to north in the northern hemisphere and from north to south in the southern hemisphere. Because the warmer weather and growth seasons also move in the same direction, the pathogens constantly find plants in their susceptible stage as the season progresses.

In each new location, however, the same set of favorable moisture, temperature, and wind or vector conditions must be repeated so that infection, reproduction, and dispersal of the pathogen can occur as quickly as possible. Furthermore, these conditions must be repeated several times within each location so that the pathogen can multiply, increasing the number of infections it causes on the host plants. These repeated infections usually result in the destruction of almost every plant within the area of an epidemic (Fig. 8-20), although the uniformity of the plants and the size of the area of cultivation, along with the prevailing weather, determine the final spread of the epidemic.

Fortunately, the most favorable combinations of conditions for disease development do not occur very often over very large areas; therefore, spectacular plant disease epidemics that destroy crops over large areas are relatively rare. However, small epidemics involving the plants in a field or a valley occur quite frequently. With many diseases, e.g., potato late blight, apple scab, and cereal rusts, the environmental conditions seem usually to be favorable, and disease epidemics would occur every year were it not for the control measures (chemical sprays, resistant varieties, and so on) employed annually to avoid such epidemics.

MODELING OF PLANT DISEASE EPIDEMICS

An epidemic is a dynamic process. It begins on one or a few plants and then, depending on the kind, magnitude, and duration of environmental factors that influence the host and pathogen, increases in severity and spreads over a larger geographic area until it finally dies down. Epidemics come to a stop when all host plants are killed by the pathogen, become resistant to the pathogen as they age, or are harvested. In many cases, epidemics slow down or come to a stop when the weather turns dry or unseasonably cold. In many ways, the appearance, development, and spread of epidemics resemble those of hurricanes. In both cases, humans have been extremely interested in determining the elements and conditions that initiate each, the conditions that influence the rate of increase and the direction of their path, and the conditions that bring about their demise. For both phenomena, observations, measurements, mathematical formulas, and computers are used extensively to study the development and to predict the size, path, and time of attack in any given location.

Each plant disease epidemic, e.g., of stem of wheat, late blight of potato, apple scab, or downy mildew of grape, follows a predictable course in each location each year. The course of the epidemic varies with the host

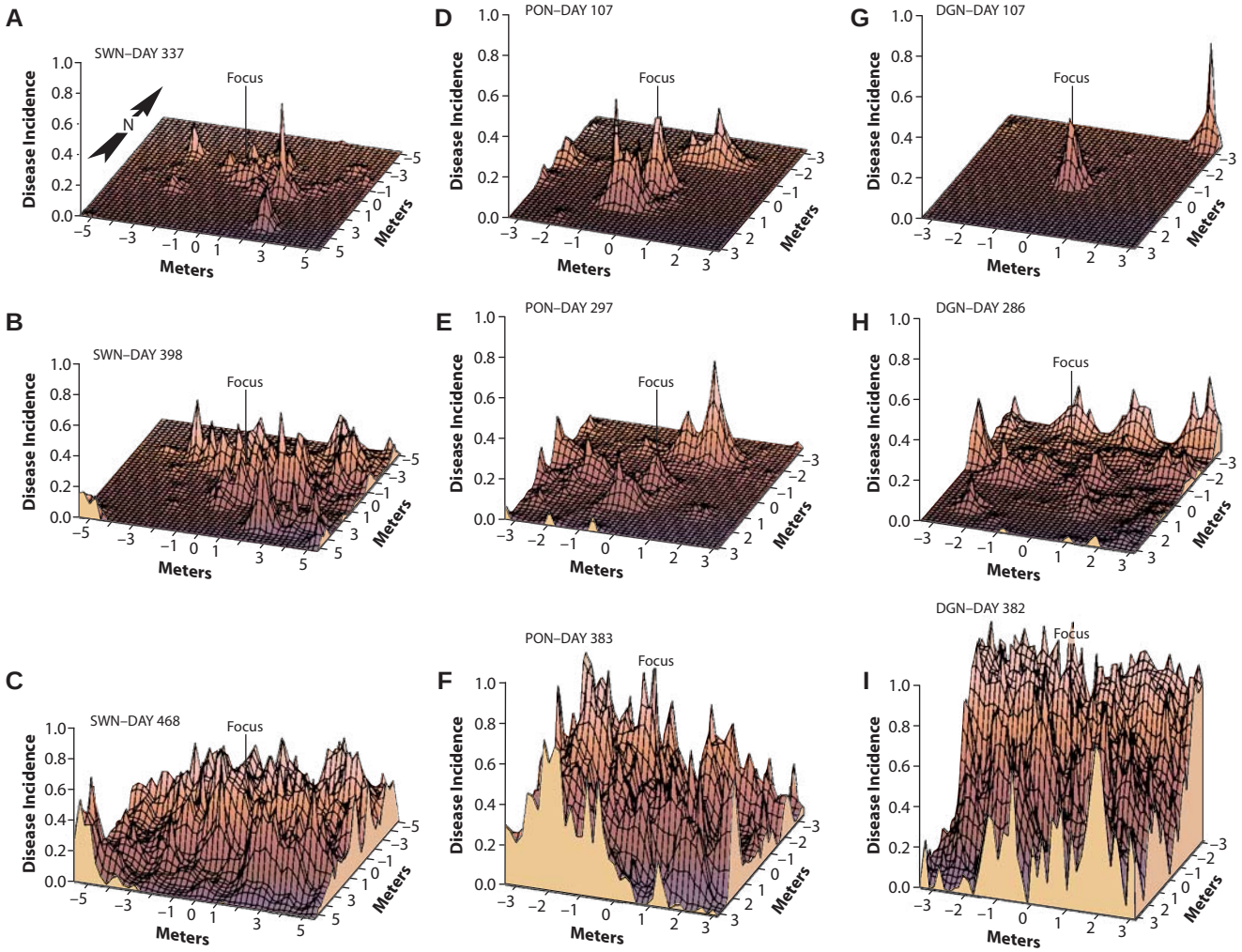

FIGURE 8-20 Development and spread of citrus canker disease, caused by the bacterium *Xanthomonas campestris* pv. *citri*, from a single inoculated plant (focus) in three citrus nurseries on the indicated days after inoculation. SWN, Swingle rootstock nursery; PON, Pinable orange nursery; DGN, Duncan grapefruit nursery. Citrus canker developed fastest in the Duncan nursery and slowest in the Swingle nursery. [From Gottwald *et al.* (1989). *Phytopathology* **79**, 1276–1283.]

varieties and pathogen races present, with the amount of pathogen inoculum present at the beginning of the epidemic, and with the moisture levels and temperature ranges during the epidemic (Fig. 8-21). The more information we have about each of the components of an epidemic and about each of its subcomponents at any given moment, the better we can understand and describe the epidemic, and the better we can predict its direction and severity at some later point in time or some other place. The ability to predict the direction and severity of an epidemic, of course, has important practical consequences: it allows us to determine whether, and when, to intervene with control measures. Moreover, it often allows us to determine what types of disease management strategies can be employed to slow down, or entirely prevent, the disease in a particular location.

In an effort to improve our ability to understand and predict the development of an epidemic, plant pathologists since the late 1960s have been developing models of potential epidemics of the most common and serious diseases. The construction of a model takes into account all of the components and as many of the subcomponents of a specific plant disease for which there is information for quantitative treatment, i.e., for treatment by mathematical formulas. The models constructed are generally crude simplifications of real epidemics, roughly analogous, for example, to model toy cars or airplanes as they compare to real cars and airplanes. As with model toys, however, one can get a better picture and understanding of the real thing as the model depicts more and more parts, as the accuracy of the proportions of these parts increases, and as the number of the parts

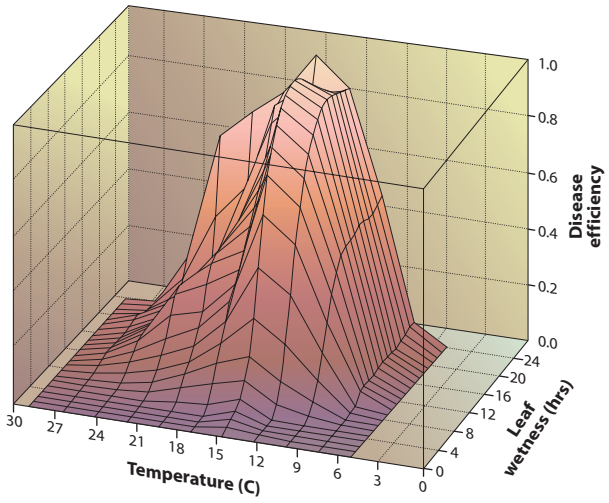

FIGURE 8-21 Model describing the effect of temperature and leaf wetness duration on the ability of the bean rust fungus *Uromyces appendiculatus* to cause disease. The maximum disease reached at 15°C and 24 hours of leaf wetness is given the maximum value of 1.0. [From Berger *et al.* (1995). *Phytopathology* 85, 715–721.]

that are interlocked and move increases. The closer the resemblance of the model to the real thing, the better we can visualize and understand the functions of the real thing by observing the model. In modeling plant disease epidemics, each component and subcomponent of the epidemic may be considered equivalent to one of the parts of the toy model; moreover, just as more accurately measured and fitted parts make for a more exact toy model, the more accurately the real subcomponents of an epidemic are measured and fitted together, the more accurately they describe the epidemic. When we have enough information about the values of the subcomponents of an epidemic at different stages and under different conditions, we can then develop a mathematical equation or equations — a mathematical model — that describes the epidemic.

Analysis of mathematical models of epidemics of specific plant diseases provides a great deal of information regarding the amount and efficacy of the initial inoculum, the effects of the environment, the disease resistance of the host, the length of time that host and pathogen may interact, and the effectiveness of various disease management strategies. Attempts to verify models of epidemics with actual observations and experimentation point out areas in which more knowledge is needed, and such analyses therefore indicate the directions in which further studies of the particular disease should be pursued.

In developing a plant disease model, a database of information is developed about as many of the components of a plant disease as possible. The database contains information on the crop, the disease, the pathogen, the location of the weather station, and sensor(s) relative to the crop and the crop canopy. The database also contains information on the input variables such as measured environmental variables, including temperature, precipitation, relative humidity and leaf wetness; calculated environmental variables such as degree hours or dew points; host variables such as crop growth stage, variety, and other host factors; and pathogen variables such as inoculum potential, spore maturity, and other pathogen factors. The mathematic relationship that describes the interaction between the environment, host and pathogen variable, and the disease is described as the model and is presented as an equation, as a graph, as a table, or as a simple statement. The information, if available, is obtained from the literature, or else it is developed through experimentation. Because plant disease models are developed for specific climates and regions, a model not developed in a specific area must be tested and validated for the specific location for one or more seasons to verify that it will work in this location.

COMPUTER SIMULATION OF EPIDEMICS

The availability of computers has allowed plant pathologists to write programs that allow the simulation of epidemics of the most important plant diseases. One of the first computer simulation programs, called EPIDEM, was written in 1969 and resulted from modeling each stage of the life cycle of a pathogen as a function of the environment. EPIDEM was designed to simulate epidemics of early blight of tomato and potato caused by the fungus *Alternaria solani*. Subsequently, computer simulators were written for Cercospora blight of celery (CERCOS), for *Mycosphaerella* blight of chrysanthemums (MYCOS), for southern corn leaf blight caused by *Cochliobolus (Helminthosporium) maydis* (EPICORN), and for apple scab caused by *Venturia inaequalis* (EPIVEN). A more general and more flexible plant disease simulator, called EPIDEMIC, was written primarily for the stripe rust of wheat but could be modified easily for other host–pathogen systems. Computer simulation programs are now available for numerous plant diseases.

In a computer simulation of an epidemic, the computer is given data describing the various subcomponents of the epidemic and control practices at specific points in time (such as at weekly intervals). The computer then provides continuous information regarding not only the spread and severity of the disease over time, but also the final crop and economic losses likely to be caused by the disease under the conditions of the epidemic as given to the computer.

Computer simulation of epidemics is extremely useful as an educational exercise for students of plant pathology and also for farmers so that they can better understand and appreciate the effect of each epidemic subcomponent on the final size of their crop loss. Computer simulations of epidemics are, however, even more useful in actual disease situations. There, they serve as tools that can evaluate the importance of the size of each epidemic subcomponent at a particular point in time of the epidemic by projecting its effect on the final crop loss. By highlighting the subcomponents of an epidemic that are most important at a particular time, the simulation serves to direct attention to management measures that are effective against these particular epidemic subcomponents. In subsequent evaluations of the epidemic, the computer evaluates not only the current status of the disease, but also the effectiveness of the applied management measures in controlling the epidemic.

FORECASTING PLANT DISEASE EPIDEMICS

Being able to forecast plant disease epidemics is intellectually stimulating and also an indication of the success of modeling or computer simulation of particular diseases. Foremost, however, it is extremely useful to farmers in the practical management of crop disease. Disease forecasting allows the prediction of probable outbreaks or increases in intensity of disease and, therefore, allows us to determine whether, when, and where a particular management practice should be applied. In managing the diseases of their crops, growers must always weigh the risks, costs, and benefits of each of numerous decisions. For example, they must decide whether or not to plant a certain crop in a particular field. Growers must also decide whether to buy more expensive propagating stock free of virus and other pathogens or whether they can "get by" with untested stock. Quite often, growers must decide whether to plant seed of a more expensive or less-yielding but resistant variety rather than seed of a high-yielding but susceptible variety that needs to be protected by chemical sprays. Most frequently, farmers need forecasts that will help them determine whether a plant infection is likely to occur so they can decide whether to spray a crop right away or to wait for several more days before they spray. If disease forecasting allows them to wait, they can reduce the amounts of chemicals and labor used without increasing the risk of losing their crop.

To develop a plant disease forecast, one must take into account several characteristics of the particular pathogen, host, and, of course, environment. In general, for most monocyclic diseases (such as root rot of peas and Stewart's wilt of corn) and for a few polycyclic diseases that may have a large amount of initial inoculum (such as apple scab), disease development may be predicted by assessing the amount of the initial inoculum. For polycyclic diseases (such as late blight of potato) that have a small amount of initial inoculum but many infection cycles, disease development can best be predicted by assessing the rate of occurrence of the infection cycles. For diseases in which both the amount of initial inoculum and the number of disease cycles are large, e.g., beet yellows, both factors must be assessed for the accurate prediction of disease epidemics. Such assessments, however, are often difficult or impossible, and, despite considerable improvements in equipment and methods, assessments of initial inoculum or rapidity of infection cycles are seldom accurate.

Disease Diagnosis: The Key to Forecasting of any Plant Disease Epidemic

Plants in a field are rarely attacked by a single kind of pathogen. More often than not, leaf spots and blotches caused by abiotic factors or bacteria may be present along with spots and blotches caused by fungi. Such symptoms may be confused with those caused by the pathogen in question and may be difficult to diagnose accurately. Such difficulty is especially likely early in the development of a disease when accurate diagnosis is needed most for determining if a threshold for development of an epidemic has been reached and appropriate instructions for its management must be issued. Inaccurate diagnosis of the pathogen in question as being present in the crop early, while in reality it is not, will lead to premature recommendation to spray and therefore to additional and unnecessary fungicide applications. However, misdiagnosis of the real pathogen as something else of lesser importance is likely to miss the opportunity to take appropriate management measures early in the development of the epidemic and to make it much more difficult and expensive to prevent the epidemic from developing and causing serious losses.

Evaluation of Epidemic Thresholds

It is always desirable for the grower to have flexibility in timing fungicide applications according to the progress of an epidemic. In diseases characterized by numerous localized infections (foliar diseases), epidemics are generally characterized by three parameters: disease incidence in individual plants, disease incidence in individual organs (usually leaves), and disease severity (percentage infected leaf area) in leaves. These

parameters mark different phases of disease development. In the early stages of disease, disease incidence in plants may increase rapidly but disease severity on individual plants is low. In the second phase of the epidemic, i.e., disease incidence in leaves, there is a small increase in disease severity along with an increase in disease incidence in leaves. Depending on the specific disease, when a percentage (e.g., 1–50%) of plants and a percentage (e.g., 1–25%) of leaves show disease incidence, these are taken as the epidemic threshold in the first two phases of the epidemic for the application of fungicides to stop or slow the development of the epidemic. During the third phase of a disease, disease severity is likely to increase rapidly (up to 2–50% per week). During this phase, fungicides are applied according to disease severity assessment, the dictates of weather conditions (rainfall, relative humidity, temperature measured daily, and providing a daily infection value), and continue as long as there is healthy tissue on the plants that needs to be protected while the crop is not yet ready for harvest.

Evaluation of Economic Damage Threshold

Although it is fairly easy to determine the epidemic threshold, in many plant diseases the threshold for a fungicide application is reached late in the season, which results in disease severity remaining low and yield not being affected. Therefore, in order to apply fungicides only when needed, one must evaluate the tolerance level of disease severity at harvest. This tolerance level, known as economic damage threshold, is the highest disease severity level that does not decrease economic profits. The economic damage threshold is obtained by studying a disease–loss relationship of disease severity at harvest and the final value of the produce and then determining the point beyond which disease severity at harvest decreases economic profits.

Assessment of Initial Inoculum and of Disease

It is often difficult or impossible, in the absence of the host, to detect small populations of most pathogens. Inoculum propagules of soilborne pathogens, such as fungi and nematodes, are estimated after extraction or trapping from soil. Airborne fungal spores and insect vectors are estimated by trapping them in various devices.

Usually it is easier to assess the amount of inoculum present by measuring the number of infections produced on a host within a certain period of time. Even in the presence of a host, however, it is often difficult to find and measure a small amount of disease. Furthermore, in many diseases there is an incubation period during which the host is infected but shows no symptoms. Aerial photography, using films sensitive to near-infrared radiation, has made possible both earlier detection and sharper delineation of diseased areas in crop fields (due to the reduced reflectance of diseased foliage tissues that are occupied by water or pathogen cells). However, for many diseases, by the time aerial photography detects diseased areas in fields, yield loss has already occurred.

Monitoring Weather Factors That Affect Disease Development

Monitoring weather factors during a plant disease epidemic presents enormous difficulties. Difficulties arise from the need for the continuous monitoring of several different factors (temperature, relative humidity, leaf wetness, rain, wind, and cloudiness) at various locations in the crop canopy or on plant surfaces in one or more fields. In the past, measurements were made with mechanical instruments that measured these environmental variables roughly or infrequently and recorded data inconveniently as ink traces on chart paper. Since the 1970s, however, several types of electronic sensors have been developed that produce electrical outputs recorded easily by computerized data loggers. Such computerized sensors are now prevalent in parts of the United States and of other countries and have improved studies of weather in relation to disease greatly and have facilitated the acceptance and use of predictive systems for disease control on the farm.

In most parts of the world, however, several types of traditional and battery-operated electrical instruments are used to measure various weather factors. Temperature measurements are made with various types of thermometers, hygrothermographs, thermocouples, and especially with thermistors (the latter are semiconductors whose electrical resistance changes considerably with temperature). Relative humidity measurements are made with a hygrothermograph (which depends on the contraction and expansion of human hair in relation to relative humidity changes), with a ventilated psychrometer (consisting of a wet and dry bulb thermometer or a wet and dry thermistor), or with an electrode-bonding sulfonated polystyrene plate (whose resistance changes logarithmically with relative humidity). Leaf wetness is monitored with string-type sensors that constrict when moistened or slacken when dry and either leave an ink trace in the process or close or break an electrical circuit. Several types of electrical wetness sensors are available that can be either clipped onto leaves or placed among

the leaves; they detect and measure the duration of rain or dew because either of the latter helps close the circuit between two pairs of electrodes. Rain, wind, and cloudiness (irradiance) are still measured by traditional instruments (rain funnels and tipping-bucket gauges for rain, cups and thermal anemometers for wind speed, vanes for wind direction, and pyranometers for irradiance). Several of these instruments, however, have become adapted for electronic monitoring.

In modern weather-monitoring systems, the weather sensors are connected to data-logging devices. Data may be read on a digital display or be transmitted to a cassette tape recorder or a printer. From the cassette, data may be transferred to a microcomputer. There they may be viewed, processed in several computer languages, organized into separate matrices for each weather variable, plotted, and analyzed. Depending on the particular disease model used, accurate weather information provides the most useful basis to predict sporulation and infection and therefore provides the best warning to time disease management practices, such as the application of fungicides.

The cost of purchase of automated weather systems (AWS) and the required time for operation and maintenance discourage their use by individual farmers, leading to the development of low-cost, automated weather instruments or stand-alone packages or to the creation and sharing of regional automated weather systems.

NEW TOOLS IN EPIDEMIOLOGY

The study of plant disease epidemiology has been facilitated greatly by new methods and new equipment that make possible studies of aspects of plant disease that were impossible or very difficult to study earlier. Some of the equipment and instruments that have contributed to modern epidemiology have been listed already. Some of the methods and other equipment that have been used to great advantage in plant disease epidemiology include the following.

Molecular Tools

The most important of these are the development and use of genetic (DNA) probes that allow the definitive detection and identification of a plant pathogen within or on the surface of a plant tissue, in a mixture with other microorganisms, and even in the vicinity of the host plant. The detection and identification of a pathogen by its genetic probe, however, are made immensely more effective through the use of the polymerase chain reaction (PCR) technique, which amplifies greatly a specific fragment of DNA present on a probe and produces millions of copies of it. These copies are then abundant enough to be detected, identified, and studied by conventional or other molecular tools. Random amplified polymorphic DNA (RAPD) markers are often used to detect genetic similarities among pathogenic strains known to show genetic heterogeneity and can also be used easily for designing sequence characterized amplified region (SCAR) markers for detecting the pathogen in infected plant tissue. The significance of the contributions of these, and some other, molecular techniques in epidemiology lies in the fact that they can detect pathogen arrival much earlier than could be detected before, thereby allowing the grower time to get ready and to apply whatever management treatment is most effective against the pathogen. Moreover, these techniques can detect any new mutant pathogens early that could either attack plant varieties they could not attack before or they may tolerate the fungicide to which they were sensitive before and thus produce a new resistant race. Detection of such changes in pathogens is of paramount importance in epidemiology because such changes in pathogens make useless and necessitate immediate revision of any previous predictions about the development of the epidemic and recommendations for management of the disease.

Geographic Information System

The geographic information system (GIS) is a computer system that can be installed on any recent model desktop computer and is capable of assembling, storing, manipulating, and displaying data that are referenced by geographic coordinates. GIS is adaptable to operations of any size, and data can be used at any scale from a single field to an agricultural region. It is used to better understand and manage the environment, including the understanding and management of plant disease epidemics. GIS techniques allow one to make connections between events based on geographic proximity, connections that are essential to the understanding and management of epidemics but which often go unrecognized without GIS. GIS techniques can even incorporate disease forecasting systems, although the time and cost for it may be prohibitive. However, as high-resolution weather forecast data are often available, the development of plant disease epidemics can be predicted by knowing their dependency on some critical weather variable and from estimated geographic distribution of the pathogen inoculum within a GIS framework. GIS is often used for the spatial and temporal analysis of disease development over relatively large geographic areas and helps

determine the role and relative importance of various parts of these areas in the initiation and development of the epidemic.

Global Positioning System

The global positioning system (GPS) consists of a hand-held device that is coordinated with a global system of man-made satellites and, depending on the accuracy and coordination, provides quite accurate readings of the coordinates of the position of the device. GPS enables one to pinpoint an individual tree or a specific area or areas of the field that are affected by a pathogen, which then can be visited and examined again periodically for incremental advance of the symptoms. Similarly, the selected trees or areas could be treated with the appropriate pesticide or other treatment wherever the pathogen is present without the need to treat the entire field. GPS can also be used to apply pesticides, plant nutrients, and so on in only the areas of the field that are infested with the pathogen or in areas deficient in a particular micro- or macronutrient. Elimination of the pathogen from the field by early detection and treatment is often effective in not allowing the pathogen to cause an epidemic in the field and beyond.

Geostatistics

Geostatistics consist of various "geostatistical" techniques that are applied in plant disease epidemiology to characterize quantitatively spatial patterns of disease development or the development of pathogen populations in space and over time. These techniques have the capability to take into account the characteristics of spatially distributed variables whether they are random or systematic. In addition to being able to detect spatial connections, geostatistical techniques can also be used for studying continuous and discrete variables. Geostatistical techniques do not require as exacting asumptions of stationarity as do other spatial autocorrelation techniques. The spatial dependence or connection can be analyzed with semivariograms. The latter quantify spatial dependence by determining the variation between samples.

Remote Sensing

Remote sensing usually refers to the use of instruments for measuring electromagnetic radiation reflected or emitted from an object. The instruments record reflected or emitted radiation in the ultraviolet, visible, or infrared part of the spectrum. The instruments used for remote sensing may be hand-held, ground-based cameras with films and filters, digital cameras, video systems, and radiometers or they may be carried on balloons, aircraft, and satellites. The various remote-sensing instruments store data obtained from field situations, and data are then printed out and are analyzed directly or by transferring them to a computer and creating visual images of data (Fig. 8-22).

Image Analysis

Image analysis refers to photography and electronic image analysis, usually of large areas of fields or of mountains. The images or photographs are taken through aerial photography, ground-based sensor data, and satellite-borne and airborne sensors. Airborne mul-

FIGURE 8-22 An epidemic of sudden death of oak caused by *Phytophthora ramorum* in California as seen by aerial photography. (Photograph courtesy of P. Svihra, University of California.)

tispectral scanning is studied and used widely for the surveillance of plant diseases, pests, and environmental stresses in agriculture. Often, infrared light or light of other wavelengths is used for the detection of the onset and progress of a plant disease among the crop plants in the field or among the fruit or forest trees in a mountain. Plants and trees, when infected with various pathogens or subjected to other stresses, turn light green, then chlorotic (yellowish), and then brown and have different reflectances from the healthy plant. These colors or shades of colors become more distinct when photographed with the wavelengths mentioned previously than when photographed with the normal visible light spectrum. More importantly, such photographs can be examined and analyzed with specific equipment that not only better distinguishes such disease-discolored plants, but can also provide a count of the newly infected plants as well as measure the changes in intensity of the images of previously diseased plants. In that way, image analysis can provide a measure of the severity of the disease in each plant or area of infected plants and, by repetition of the photography at regular intervals, provide a measure of the rate of progress of the disease.

Information Technology

This technology involves primarily the use of computers alone or in combination with other electronic devises. They help collect data on plant diseases at various levels and various locations in a continuous manner. Data are either stored or are organized, integrated, and analyzed in tremendous quantities and at hitherto unimaginable speeds and eventually are used to produce visual images or written reports and recommendations. Electronic information technology can, above all, describe and display spatial patterns of characteristics of different pathogens, such as their genotypes, at the scale of an agricultural region.

EXAMPLES OF PLANT DISEASE FORECAST SYSTEMS

Generally, it is useful to have the maximum amount of information that is available about a disease before venturing to predict its development. In many cases, however, one or two of the factors that affect disease development predominate so much that knowledge of them is often sufficient for the formulation of a reasonably accurate forecast. Thus, forecasting systems of several plant diseases use the amount of the initial inoculum as the criterion. Such diseases include Stewart's wilt of corn, blue mold of tobacco, fire blight of apple and pear, pea root rot, and other diseases caused by soilborne pathogens such as *Sclerotium* and cyst nematodes. Forecasting systems of diseases such as the late blight of potato, *Cercospora* and other leaf spots and the downy mildew of grape use the number of infection cycles or the amount of secondary inoculum as the criterion. Forecasting systems of still other diseases, e.g., apple scab, black rot of grape, cereal rusts, *Botrytis* leaf blight and gray mold, and sugar beet yellows, use the amount of the initial inoculum and the number of infection cycles or the amount of secondary inoculum as criteria.

Forecasts Based on Amount of Initial Inoculum

In Stewart's wilt of corn [caused by the bacterium *Erwinia (Pantoea) stewartii*], the pathogen survives the winter in the bodies of its vector, the corn flea beetle. Therefore, the amount of disease that will develop in a growing season can be predicted if the number of vectors that survived the previous winter is known, as that allows an estimation of the amount of inoculum that also survived the previous winter. Corn flea beetles are killed by prolonged low winter temperatures. Therefore, when the sum of the mean temperatures for the three winter months December, January, and February at a given location is less than –1°C, most of the beetle vectors are killed and so there is little or no bacterial wilt during the following growth season. Warmer winters allow greater survival of beetle vectors and proportionately more severe wilt outbreaks the following season.

In the downy mildew (blue mold) of tobacco (caused by the oomycete *Peronospora tabacina*), the disease in most years is primarily a threat to seedbeds in the tobacco-producing states. When January temperatures are above normal, blue mold can be expected to appear early in seedbeds in the following season and to cause severe losses. However, when January temperatures are below normal, blue mold can be expected to appear late in seedbeds and to cause little damage. If the disease is expected in seedbeds, control measures can be taken to prevent it from becoming established, and subsequent control in the field is made much easier. Since 1980, a supplementary blue mold warning system has been operated in North America by the Tobacco Disease Council and the Cooperative Extension Service. The warning system keeps the industry aware of locations and times of appearance and spread of blue mold and helps growers with the timing and intensity of controls.

In pea root rot (caused by the oomycete *Aphanomyces euteiches*) and in other diseases caused by soilborne fungi and some nematodes, the severity of the disease in

a field during a growing season can be predicted by winter tests in the greenhouse. In these tests, susceptible plants are planted in the greenhouse in soil taken from the field in question. If the greenhouse tests show that severe root rot develops in a particular soil, the field from which the soil was obtained is not planted with the susceptible crop. However, fields whose soil samples allow the development of little or no root rot can be planted and can be expected to produce a crop reasonably free of root rot. With some soilborne pathogens, such as fungi *Sclerotium* and *Verticillium* and the cyst nematodes *Heterodera* and *Globodera*, the initial inoculum can be assessed directly by isolating the fungal sclerotia and nematode cysts and then counting them per gram of soil. The greater the number of propagules, the more severe the disease produced.

In fire blight of apple and pear (caused by the bacterium *Erwinia amylovora*), the pathogen multiplies much more slowly at temperatures below 15°C than at temperatures above 17°C. In California, a disease outbreak can be expected to occur in the orchard if the daily average temperatures exceed a "disease prediction line" obtained by drawing a line from 16.7°C on March 1 to 14.4°C on May 1. Therefore, when such conditions occur, application of a bactericide during bloom is indicated to prevent an epidemic.

Forecasts Based on Weather Conditions Favoring Development of Secondary Inoculum

In late blight of potato and tomato (caused by the oomycete *Phytophthora infestans*), the initial inoculum is usually low and generally too small to detect and measure directly. Even with low initial inoculum, the initiation and development of a late blight epidemic can be predicted with reasonable accuracy if the moisture and temperature conditions in the field remain within certain ranges favorable to the fungus. When constant cool temperatures between 10 and 24°C prevail and the relative humidity remains over 75% for at least 48 hours or is at least 90% for 10 hours each day for 8 days, infection will take place and a late blight outbreak can be expected from 2 to 3 weeks later. If, within that period and afterward, several hours of rainfall, dew, or relative humidity close to the saturation point occur, they will serve to increase the disease and will foretell the likelihood of a major late blight epidemic (Fig. 8-23).

Computerized predictive systems have been developed for epidemics of late blight and several other diseases; in some such systems, e.g., BLITECAST for late blight (Fig. 8-20); FAST (for forecasting *Al. solani* on tomatoes); TOMCAST (for tomato forecaster) for tomato early blight, *Septoria* leaf spot, and anthracnose; and PLAM for peanut leaf spot, moisture and temperature are monitored continuously. From this information weather severity values are calculated, infection and disease severity values are predicted, and recommendations are issued to growers as to when to begin spraying. More recent refinements in late blight forecasting include, in addition to data on moisture and temperature, information on the level of resistance of the potato variety to late blight and the effectiveness of the fungicide used. Information on all these parameters is, of course, very useful in the formulation of recommendations for fungicide applications.

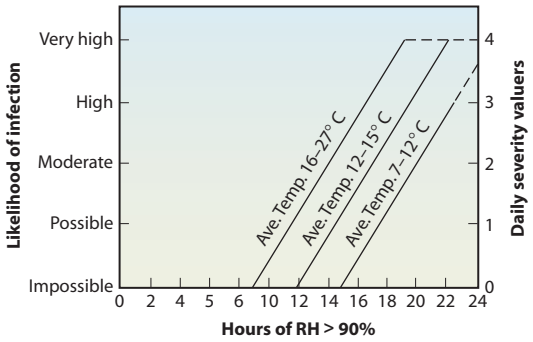

FIGURE 8-23 Relationship of the duration of high relative humidity periods and average temperature during such periods to the likelihood of potato infection by the late blight fungus *Phytophthora infestans*. The daily severity values are arbitrary values given by the relative humidity–temperature relationship; they correspond to the likelihood of infection shown at left and are used to recommend spray schedules with BLITECAST. [From MacKenzie (1981). *Plant Dis.* 65, 394–399.]

Several leaf spots, such as those caused by the fungi *Cercospora* on peanuts and celery and *Exserohilum* (*Helminthosporium*) *turcicum* on corn, can be predicted by taking into account the number of spores trapped daily, the temperature, and the duration of periods with relative humidity near 100%. An infection period is predicted if high (95–100%) relative humidity lasts for more than 10 hours, and growers are then urged to apply chemical sprays immediately.

Forecasts Based on Amounts of Initial and Secondary Inoculum

In apple scab (caused by the fungus *Venturia inaequalis*), the amount of initial inoculum (ascospores) is usually large and is released over a period of 1 to 2 months following bud break. Infections from the primary inoculum must be prevented with well-timed fungicide applications during blossoming, early leafing, and fruit development; otherwise, the entire crop is likely to be lost. After primary infections, however, secondary

inoculum (conidia) is produced, which multiplies itself manyfold with each succeeding generation. The pathogen can infect wet leaf or fruit surfaces at a range of temperatures from 6 to 28°C. The length of time that leaves and fruit need to be wet, however, is much shorter at optimum temperatures than at either extreme (9 hours at 18–24°C versus 28 hours at 6 to 28°C). By combining temperature and leaf wetness duration data, the apple scab forecast system can predict not only whether an infection period will occur, but also whether the infection periods will result in light, moderate, or severe disease (Fig. 8-24). Such information, collected and analyzed by individuals or by weather-sensing microcomputers, is used to make recommendations to growers. The latter are advised of the need and timing of fungicide application and about the kind of fungicide (protective or eradicant) that should be used to control the disease.

In wheat leaf and stem rusts (caused by fungi *Puccinia recondita* and *Puccinia graminis*), short (1–2 week) forecasts of subsequent disease intensity can be obtained by taking into account disease incidence, stage of plant growth, and spore concentration in the air.

In many insect-transmitted virus diseases of plants (e.g., barley yellow dwarf, cucumber mosaic virus, and sugar beet yellows), the likelihood, and sometimes the severity, of epidemics can be predicted. This is accomplished by determining the number of aphids, especially viruliferous ones, coming into the field at certain stages of the host growth. A number of the aphids caught in traps placed in the field are tested for virus by allowing them to feed on healthy plants or by analyzing them for virus serologically with the ELISA technique or with nucleic acid probes. The more numerous the viruliferous aphids and the earlier they are detected, the more rapid and more severe will be the virus infection. Such predictions can be improved by taking into account late winter and early spring temperatures, which influence the population size of the overwintering aphid vectors.

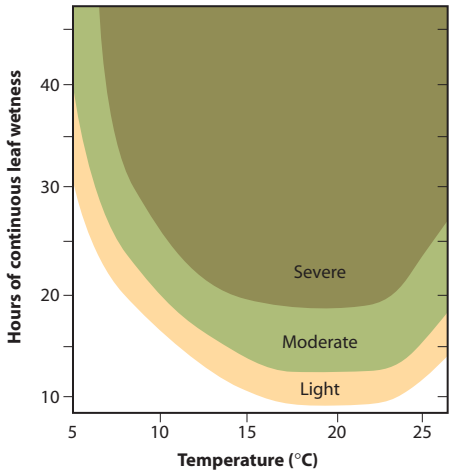

FIGURE 8-24 Relationship of temperature and duration of leaf wetness to the occurrence and severity of apple scab caused by the fungus *Venturia inaequalis*. [From Mills (1944). *Cornell Ext. Bull.* 630.]

RISK ASSESSMENT OF PLANT DISEASE EPIDEMICS

The risk of development of a plant disease into an epidemic is the probability that a certain intensity of incidence or severity of the disease will be reached. For example, a possible risk of tomato early blight can be estimated as 10% incidence with 85% probability. However, the risk of plant disease can also be determined as the probability, e.g., 90%, that the maximum possible incidence of a disease being about 60%, will not be reached. Numerous host, pathogen, and environmental factors must be taken into account in assessing the risk of development of a particular plant disease: history of the disease in the field from previous years, resistance of planted varieties, presence and amount of primary inoculum, period of susceptibility of the host, prevailing weather conditions (temperature, rainfall, relative humidity) during periods of susceptibility, availability and cost of effective control measures, and so on. Since in most cases information on all of these parameters remains fairly constant from year to year, one needs to concentrate primarily on estimating as well as possible the starting inoculum of the pathogen and, subsequently, in following closely changes in temperature and moisture, appearance of first signs of the disease in the field, and predictions of weather changes in the near future. When all the parameters, constant and variable (temperature, rainfall, relative humidity), are known, or estimated from the best data available, a knowledgeable person can project with some certainty the likely risk of the disease developing up to a certain level of severity. Risk assessment is sometimes expressed as percentages of obtaining certain values of disease severity; more often, however, it is expressed as low, moderate, or high risk of reaching those disease severity values. Nevertheless, risk assessment provides a timely warning to the grower who subsequently responds with appropriate urgency in applying effective and sufficient management measures.

DISEASE-WARNING SYSTEMS

In many states and countries, different types of warning systems are in place for one or more important plant

diseases. The purpose of these systems is to warn farmers of the impending onset of an infection period or to inform them that an infection period has already occurred so that they can take immediate appropriate control measures to stop recent infections from developing or prevent further infections from occurring.

In most cases, the warning system begins with a grower, an extension agent, or a private consultant surveying certain fields on a regular basis or when the weather conditions are likely to favor maturation of the primary inoculum or appearance of the particular disease. When mature inoculum (such as ascospores in apple scab) or traces of disease (e.g., in potato late blight) are found, the county extension office is notified. The county extension office in turn notifies the state extension plant pathologist, who collates all reports about the disease from around the state and by electronic mail (e-mail), telephone, fax, or in writing notifies all concerned county agents (pest alert). They, in turn, by e-mail, radio, telephone, or letter, notify all farmers in their county. For diseases of potential regional or national epidemic consequences, the state extension plant pathologist notifies the federal plant disease survey office of the U.S. Department of Agriculture, which in turn notifies all extension plant pathologists in adjacent and other states that may be affected by that plant disease.

Since the mid-1970s, computerized warning systems have been in use for certain diseases in some states. Some of them (such as BLITECAST) use centrally located computers that process weather data either collected on the farm by individual growers and transmitted electronically or phoned in when certain weather conditions prevail, or at certain intervals. The computer then processes the data, determines whether an infection period is imminent, likely to occur, or cannot occur, and makes a recommendation to the grower as to whether to spray and what materials to apply.

After 1980, small special-purpose computers have been used that have field sensors and can be mounted on a post in a farmer's field. Such units (such as the apple scab predictor) monitor and collect data in the field on temperature, relative humidity, duration of leaf wetness, and rainfall amounts, analyze the data automatically, make predictions of disease occurrence and intensity, and, on the spot, make recommendations for disease control measures. The same unit can be used for any disease for which a prediction program is available, in which case either the unit can be reprogrammed or the program circuit boards can be interchanged. Predictions from such units are obtained by using a simplified keyboard and display right in the field or the unit can be linked to a personal computer if additional processing of data is desired.

DEVELOPMENT AND USE OF EXPERT SYSTEMS IN PLANT PATHOLOGY

Expert systems are computer programs that try to equal and, better yet, surpass the logic and ability of an expert professional in solving problems, the solutions of which require experience, knowledge, judgment, and complex interactions. The dependability of an expert system is proportional to the knowledge of the expert(s) who produced it. Expert systems can use data in almost any format and can suggest a solution to the problem; they can even use incomplete or incorrect data, as long as the degree of certainty of data is quantified by the expert and is included in the knowledge base. Expert systems in plant pathology are used frequently for diagnostic purposes, i.e., identifying the cause of a disease by the symptoms and related observations. Several expert systems, however, incorporate the decision-making process of the expert and advise producers in making disease management decisions. By incorporating infection models of the important diseases of a crop into the knowledge base of the computer, the expert system can advise growers of disease potentials on the basis of the actual occurrence of infection periods and provide pesticide recommendations and suggestions for pesticide amounts and timing of application.

The development of even simple expert systems is quite complex, but advances in computers and increasing familiarity with their use are making the development and use of expert systems increasingly attractive. In their simplest form, expert systems utilize a bank of data pertinent to the problem stored in the computer and also a knowledge base inputed by the expert(s) and consisting of one or more "IF conditions" followed by a conclusion or action (THEN action) and, finally, a recommendation. In addition to the requirement of being familiar with computer programming, the most important part of creating an expert system is the quality (expertise) of the expert(s) providing the knowledge that is inputed in the system. This knowledge of the expert(s) is then represented in a form that can be converted into computer code. Once a prototype expert system is generated, it is first tested for logic and accuracy. Usually, the expert system is also reviewed and, if necessary, revised by other experts; subsequently, it is tested with the intended users, and additional revisions are made before the expert system is released for use. Even after an expert system is released to its final users, it must be revised and updated regularly.

BLITECAST (1975), which is a computerized forecasting system for potato late blight, and the computer-based apple scab predictive system (1980) are considered to be the precursors to expert systems. The first expert system in plant pathology was developed in

1983 to diagnose nearly 20 soybean diseases in Illinois. Since then, expert systems have been developed for the diagnosis or management of diseases of tomato (TOM), grape (GrapES), wheat (CONSELLOR), peach and nectarine (CALEX), apple (POMME, the Penn State apple orchard consultant PSAOC), wheat (MoreCrop), and others.

An example of an "expert" advisory system is MoreCrop, which stands for "Managerial Options for Reasonable Economical Control of Rusts and Other Pathogens." MoreCrop is designed to provide disease management options in different geographic regions and agronomic zones of the Pacific Northwest using the vast information available on wheat diseases as well as advances in computers. The components of MoreCrop and their functional relationships are understood. Some of the frames ("windows") of the program show the wheat diseases about which one should be concerned. Brief information about each disease, suggestions for disease control through seed treatment and foliar spray, timing of sprays, spray label restrictions, and which diseases can or cannot be controlled through a particular treatment are shown in relevant frames.

Expert systems are used primarily, but not exclusively, with high value horticultural crops that require frequent application of pesticides as part of their disease and pest management, usually in response to site-specific weather conditions. Although expert systems are aimed for use by growers of such crops, they are also used by individuals, such as county agents and pesticide distributors, who influence grower decisions.

DECISION SUPPORT SYSTEMS

A fully developed decision support system (DSS) is supposed to collect, organize, and integrate all types of information related to the production of a crop, to subsequently analyze and interpret the information, and to eventually recommend the most appropriate action or action choices. Decision support systems for plant disease management may be very simple, e.g., a data processing device, fairly complex, e.g., a computerized expert system, or extremely complex, including automated weather and combinations of decision aids and expert systems, as well as multidisciplinary teams of knowledge specialists. Numerous DSS systems available are aimed to assist practitioners in the field, including county agents, crop consultants, growers, and others. Many of them have plant disease management modules, such as WISDOM for potatoes by the University of Wisconsin, RADAR for apples by the University of Maine, PAWS for several crops by the Washington State University, and another one, Fieldwise.com, used on several crops on the west coast. Of the many available DSS systems, relatively few are used because they address only specific disease problems, they are too complex to operate, or for other reasons. Cooperation among universities, growers, and industry has resulted in the development of the Penn State apple orchard consultant in the United States, while in Australia, development of the AusVit DSS for grapes came about through the cooperation of several state departments of agriculture, universities, grower organizations, and private industry. It is apparent that the development and usage of DSS will become more regional rather than local. The continuing demise of the family farm and the increase in large farms, however, are expected to increase the use of DSS systems significantly.

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